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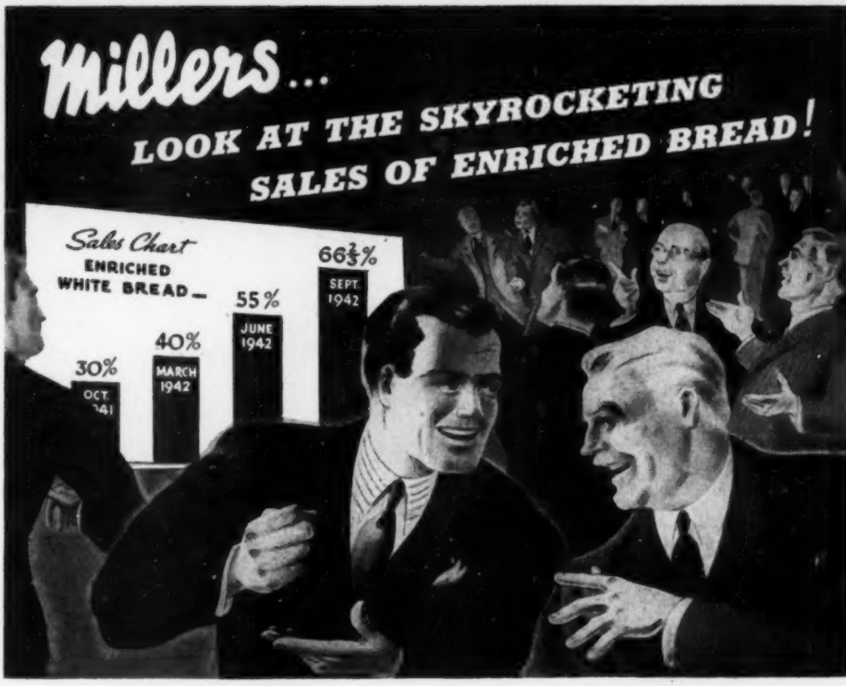

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Memorial

TO

CARL LUCAS ALSBERG

1877–1940

CARL LUCAS ALSBERG, 1877–1940

CEREAL CHEMISTRY

Vol. XIX NOVEMBER, 1942 No. 6

CARL LUCAS ALSBERG

CARL LUCAS ALSBERG died suddenly on October 31, 1940, at Berkeley, California, in his 64th year. Students of the natural and social sciences alike, all over the world, have lost a farseeing leader, a wise counselor, and an energetic and co-operative fellow-worker.

Alsberg was born in New York City in 1877, the eldest of four children of German-Jewish stock in a middle-class family of intellectually liberal tradition. The circumstances were comfortable rather than affluent. The boyhood environment, as his friend Alfred Kroeber has well said, gave him "the rare and valuable attitude of being every man's inherent equal and no one's superior; a genuine ease and warmth, supplemented by his native kindliness, in relations with people; an ability to subordinate himself to the majority, or circumstances, without withdrawing his independence of private judgment; and an even more outstanding faculty of directing others with their full loyalty and approval."

His early education was in private schools and by tutors, one of whom he was fond of citing as a master of pedagogy: Alsberg never forgot his first lesson in geography, taught not from a book but from the vantage point of Brooklyn Bridge. He entered Columbia University at the age of fifteen and proceeded after graduation to the College of Physicians and Surgeons, taking the degree of M.D. at the age of twenty-three. The practice of medicine, however, held lesser attractions than inquiry into things unknown. Yet the training in medicine stood him in good stead. Frequently in later years he referred with special satisfaction to the instruction he had received in psychiatry, whence sprang solution of personnel problems otherwise possibly baffling to administrators. Three years of his life after graduation from medical school were spent in Germany in study of the newly unfolding field of biochemistry. They were years made the more fruitful by his upbringing in a completely bilingual family where German was spoken as often as English.

From 1902 to 1908 Alsberg served successively as Assistant, Instructor, and Head in Physiological and later Biological Chemistry at the Harvard Medical School. In 1908 he entered the government

service, first (until 1912) as Chemical Biologist in the Bureau of Plant Industry; next (until 1921) as Chief of the Bureau of Chemistry, United States Department of Agriculture. His marriage to Emma Blount Peebles, who survives him, occurred in 1912. He returned to academic life in 1921, as one of three Directors of the Food Research Institute at Stanford University. In 1938, as he approached the retirement age of sixty-five at Stanford, he resigned to become Director of the Giannini Foundation of Agricultural Economics at the University of California, where the university statutes allowed him five additional years of prospective activity.

Particularly in the closing decade of his life, he gave generously of his time and strength to participation and counsel beyond the routine of his academic appointments. He long served as Dean of Graduate Study at Stanford, and was a leading contributor to the activities of the Institute of Pacific Relations, the Social Science Research Council and its Pacific Coast Regional Committee, the Commission of Inquiry into National Policy in International Economic Relations, and the Board of Regents of Reed College.

Alsberg was a genuinely modest man. Speaking in 1938 to the graduating class at Reed College, he said (and meant): "I don't see anything in my career important enough to hold your attention for half an hour. . . . Life has treated me better than I deserve. . . . In me you see a Jack of many trades, a master of none. . . . Physician, biochemist, teacher, chemist, administrator, economist—I've had a fling at all of them in turn. It's been fun, but it hasn't made a great man of me. . . ."

It was fun in the sense that it made a career which sprang from and gave full scope for the two dominant aspects of his character—insatiable curiosity and indifference to financial success. Alsberg could have been a successful and wealthy physician, a great biochemist, a rich and famous consulting chemist. He chose not to cramp himself and not to specialize, but to blaze trails in unexplored country; not to become rich, but to live modestly. The choice was conscious and deliberate.

Although he chose to plow widely where he might have made deeper furrows, his plowing was by no means shallow. To all of his fields of inquiry he made genuine contributions. In the branches of natural science, his papers on the chemistry of proteins, on toxic substances, and on the physical and chemical properties of the starches have withstood the test of time. So also with his work in the social sciences, on the American baking industry, the fats and oils, population problems, relationships of weather to wheat yields, standards of living, and international relations.

Underlying Alsberg's breadth of interests and activities was his basic concern with a fundamental human problem, that of synthesizing cold fact with pure theory, progress in pure science and technology with progress in social science and human welfare. Possession of his peculiar combination of attitude and aptitude for this task is rare. Among the many students and associates of his varied career, it evoked affection and inspired respect and emulation in a degree accorded to few men.—M. K. Bennett.

BIOCHEMICAL PROPERTIES OF "SALT-RISING" DOUGHS [1]

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(Read at the Annual Meeting, May 1942)

The leavening of bread doughs by the gas-producing function of nonpathogenic micro-organisms other than yeasts has received only minor commercial and scientific attention in recent years. In the pioneering days of the American West, however, yeast leavening was difficult to obtain and bacterial or so-called "salt-rising" fermentation was widely used in home bread production. The ferment was prepared by incubating a thin batter made with corn meal and fresh milk containing a little sugar and sodium bicarbonate for six to ten hours at 38C. This starter was sponged with flour and incubated for an additional two to four hours, when the remaining ingredients, such as sugar, shortening, salt, and additional flour required to form the dough, were added and mixed. The dough was immediately placed in the pans and, after rising to approximately twice its original volume, it was baked in the usual manner. A baked loaf thus produced is of relatively small volume, with sharp corners and a smooth dark crust. The crumb is very close-grained and the bread has a unique aroma and taste somewhat resembling that of mild cheese.

Kohman (1912) studied salt-rising fermentation and showed that bacteria rather than yeasts were present in the fermenting sponges and doughs. He found that the bacterial flora predominating in a salt-rising starter depended on the temperature. At starter temperatures below 65°C a nonspore former, which did not liquefy gelatin, predominated and would successfully produce the bread. From the cultural characteristics given for this organism it would appear to be a strain of *Escherichia coli*. However, when corn meal starters were

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[1] Paper No. 2004, Scientific Journal Series, Minnesota Agricultural Experiment Station, condensed from a thesis presented by Max Milner to the faculty of the Graduate School in partial fulfillment of the requirements for the M.S. degree, July, 1941.

prepared at temperatures of 75°–100°C, a spore-forming gelatin-liquefying organism predominated. As a result of these studies Kohman (U. S. Patent No. 1,149,839; 1915) developed a commercial preparation known as "Kohman's salt-rising yeast" for use in the production of salt-rising bread. In preparing this leaven, a fermenting flour sponge started at 75°–100°C is dried, ground, and diluted with corn meal, sodium bicarbonate, and calcium carbonate. The preparation is described as a pure culture of a rod-shaped facultative anaerobe which forms very resistant spores, liquefies gelatin, and grows well in milk and broth. From these and other cultural characteristics, it would appear to be a member of the aerobacillus group.

In studying the production of gas by the salt-rising leaven, Kohman found that casein was the most favorable substrate, followed by milk and then corn meal. The higher the content of protein degradation products in the casein, the sooner gas production appeared, but the addition of increments of lactose was essentially without influence. The gas resulting from the fermentation contained two volumes of hydrogen per unit volume of carbon dioxide. These observations indicate that carbohydrates are not involved in the gas-producing function of the ferment. Recent studies of dissimilation of carbohydrates by bacteria, reviewed by Harden (1932), Werkman (1939), Nord (1940), and Kalckar (1941), show that the ratio of hydrogen and carbon dioxide is never markedly in excess of unity.

The salt-rising bacteria bring about extensive proteolysis and the unique characteristics of the bread are doubtless attributable to this action. No intermediate fermentation is required after the dough ingredients are mixed with the fermenting sponge. Apparently the conditioning of the gluten, brought about by fermentation and punching in the instance of yeast doughs, is effected by the bacterial proteases. The publications of Sperry and Rettger (1915), Merrill and Clark (1928), Buchanan and Fulmer (1930), Hoogerheide and Weil (1939), Stephenson (1939), and others show that coli bacteria and aerobacillae, like other heterotrophic micro-organisms, including the yeasts, require adequate sources of available nitrogen for their metabolism. However they differ from the yeasts in that they secrete both endo- and exoproteases; the latter degrade the large protein aggregates of the medium to smaller fragments, which are assimilated and acted upon by the proteases and polypeptidases within the cells.

Bacterial proteases are inhibited by acids and work best in neutral or alkaline solutions; this has led many authors to classify them with the tryptases. Hoogerheide and Weil (1939) found that the exoproteases secreted by certain spore-forming anaerobes could be markedly activated by the addition of SH compounds in the presence of

traces of heavy metals. Such bacteria have been shown to produce a large number of fatty acids from casein as well as indole and skatole and a variety of amines of the putrefactive type. These products are undoubtedly responsible for the characteristic odor and flavor of salt-rising bread.

In the years since Kohman's investigations, cereal knowledge has been extended. The proteolytic nature of the salt-rising fermentation renders it of particular interest to study the effect of potassium bromate on the physical properties of the doughs, as measured by such instruments as the Brabender farinograph and extensograph, and to compare these properties with those of doughs made with yeast. The present study comprises an investigation of the comparative physical, physico-chemical, and chemical properties of salt-rising and yeast-leavened doughs. A method was developed for the experimental production of salt-rising bread and a study made of the effect of varying such factors as starter time, absorption, milk, sugar, potassium bromate, and pH. Comparisons of the physical properties, rates of gas production, reducing sugar, acidity, and amino nitrogen content of salt-rising and yeast-leavened doughs were made in order to elucidate certain differences between bacterial and yeast fermentation.

Experimental Materials and Methods

An 83% bleached patent flour of 10.8% protein and 0.40% ash content, on a 13.5% moisture basis, commercially milled from a blend of hard red spring and hard red winter wheats for the family trade, was used throughout the studies. Kohman's "salt-rising yeast," as supplied to the bakery trade, served as a source of the salt-rising ferment, while Fleischmann's yeast was employed for the yeast-leavened doughs. The milk solids were a standard spray-dried skim-milk powder manufactured for the baking trade.

An experimental technique for the production of salt-rising bread was developed and will be detailed later. In comparative tests of salt-rising and yeast doughs, the formulas were identical except for the leavening agent.

Measurements of gas production in salt-rising sponges and doughs were made in a slightly modified form of the standard pressuremeter apparatus described in *Cereal Laboratory Methods*. To reduce the error involved it seemed desirable to employ relatively large samples of starters and sponges. Accordingly somewhat larger pressuremeters than the regular type were constructed. As a measure of precaution, these were equipped with manometers capable of recording higher pressures, although in practice the pressures were released at frequent intervals. Upon calibration it was found that readings could be con-

verted to those of the standard type by use of the factor 1.28. However, as the primary interest was in relative rather than absolute gas production, the readings were not corrected. Because of the known temperature requirements of the bacteria, the tests were carried out at 38°C.

The reducing-sugar content of leavened and unleavened doughs was determined by a modification of the Blish-Sandstedt procedure for flour diastatic activity, as outlined in *Cereal Laboratory Methods*. By means of the Waring Blendor, 50 g of dough was dispersed in the required amount of combined sulfuric acid and buffer solution to give the solids-liquid ratio employed in the regular flour procedure. After two minutes the sodium tungstate solution was added and the Blendor operated for another minute. The suspension was centrifuged, the supernatant liquid filtered, and a suitable aliquot employed for the determination of reducing sugars by the regular ferricyanide procedure. The results were expressed as milligrams of maltose per dough weight equivalent to 10 g of flour containing 15% moisture.

In determining titratable acidity and amino nitrogen content, 300 g of the sponges or doughs were disintegrated with 500 ml of distilled water containing 2 g of sodium chloride in a Waring Blendor, operated for 2.5 minutes. A portion of the suspension was centrifuged and the titratable acidity and amino nitrogen content determined in 10 ml of the supernatant liquid by a modification of the Sorenson formol titration procedure suggested by Samuel (1934) using $N/14$ NaOH solution. This procedure, fully described in *Cereal Laboratory Methods* (4th ed., 1941), involves the use of phenol-red indicator and titration to pH 8.0, as determined by matching against a buffer solution of this pH in a comparator block.

Changes in the physical properties of yeast and salt-rising doughs during fermentation were followed by means of the Brabender farinograph and extensograph. The formulas and techniques employed will be detailed in a later section.

Experimental Baking Procedure for Salt-Rising Bread

In order to study conveniently the factors affecting salt-rising bread production it was necessary to develop a baking procedure employing 100 g of flour. The first trials, with a small-scale formula based on the method recommended for bread made with Kohman's prepared ferment, resulted in failure. Fermentation occurred in the starters and sponges but very little was apparent in the doughs. In studying numerous variations in formula and technique it was found that the addition of sodium bicarbonate at the sponge stage resulted in a marked increase in loaf volume. Various concentrations of other

buffer salts were then employed and the best results were secured with dibasic sodium phosphate to maintain a higher pH. The effects of varying dosages of this buffer salt on baking properties are shown in

TABLE I

EFFECT OF DIBASIC SODIUM PHOSPHATE ON LOAF CHARACTERISTICS

Dibasic sodium phosphate[1]	Loaf volume	External loaf characteristics	Internal loaf characteristics		
			Crumb grain	Crumb color	Crumb texture
%	cc				
0.0	385	Pale, bad shell top	Dense	Gray	Hard
0.3	420	Pale, shell top	Dense	Gray	Hard
0.6	420	Pale, shell top	Dense	Gray	Harsh
1.2	550	Good color, even break	Excellent	Good	Smooth
1.5	520	Good color and break	Good	Slightly open	Good

[1] Expressed as the percentage anhydrous salt based on the total flour and milk solids on a 15% moisture basis in the finished dough.

Table I. As a result of these preliminary studies, the following basic procedure for the experimental production of salt-rising bread was adopted.

Formula

	Starter g		Sponge g		Dough g
Kohman's "yeast"	30	Flour	145	Flour	95
Milk solids (dry skim)	23	Diabasic sodium phosphate ($Na_2HPO4 . 12H_2O$)	8	Milk solids (dry skim)	3
Water	180 ml	Starter	40	Sugar	5
		Water	118 ml	Shortening (hydrogenated)	5
				Salt	3
				Sponge	Entire amount

Fermentation Schedules

Starter	Sponge	Dough
Ferment 9 hrs at 38°C	Ferment at 38°C until sponge begins to fall (1.75–3.0 hrs)	Place directly in tall form "100-g" experimental baking pans

Mix dough in the Hobart-Swanson mixer for 2 minutes. Proof 2.5 hrs at 38°C or until top of dough is level with top edges of the high sides of the pan. Bake for 25 minutes at 230°C (± 5°).

The ferment and milk solids are vigorously stirred into the water, which has been brought to a boil in a 600-ml beaker. As the ferment hydrates slowly, stirring must be continued for several minutes to disperse any lumps that may form. The beaker is covered with a watch glass and placed in the fermentation cabinet.

In preparing the sponge, the sodium phosphate is dissolved in water at 50°C in a one-liter beaker. The flour and starter are added and mixed with a large flexible spatula. The beaker is covered with

a watch glass and the sponge fermented at 38°C until it has commenced to fall.

While the sponge is fermenting, the other dough ingredients are combined and warmed to a sufficiently high temperature (approximately 45°C) to give a final dough temperature of 38°C. When the sponge is ready to take, the warmed ingredients are transferred to the bowl of the Hobart-Swanson mixer, the sponge added, and the mixer operated for 2 minutes. The dough is removed from the mixer bowl and rounded up by folding 10 times in the hands. Two 200-g portions are quickly scaled off (to avoid excessive cooling) and immediately molded by the hand or rolling pin method as directed for the regular A.A.C.C. bread-baking test. The molded doughs are placed in lightly greased pans and proofed and baked as outlined in the above schedule. Loaf volume was measured one hour after removal from the oven and the loaves scored for external and internal characteristics the following day. Loaf type was judged by referring to the photostandards of Blish (1928).

Notes: This formula differs somewhat from the recommended commercial procedure. A larger proportion of starter is used in the sponge and all the remaining water required for the dough is added at this stage; in addition, a buffer salt is employed in the sponge.

Because of the pronounced slackening of the doughs during proofing, they must be mixed at approximately 5% lower absorptions than yeast doughs.

The molding procedure does not appear to be as critical as with yeast doughs. The dough contains little or no gas and before gas production is under way during the proof, the dough settles to a smooth coherent mass in the bottom of the pans. The marked tendency of the doughs to flow during the proof period made the tall-form baking pans desirable. These pans provided greater support for the mobile doughs than the low-form pans and gave greater differentiation in crust character, break, and shape.

In studying the effect of absorption and potassium bromate, useful information was obtained by proofing one of the duplicate doughs to height and the other to time. Thus an increase in absorption shortened, and potassium bromate lengthened, the time required to proof to height.

The sensitivity of the doughs to under- or over-fermentation was manifested principally in the loaf type, which ranged from pale shell-topped loaves to loaves with dark crusts and no break.

The procedure was found to give consistent and readily reproducible results both within and between days. In the majority of cases the volumes of duplicate loaves checked within 5 cc.

Effect of Variations in Formula and Procedure on Baking Properties

With the basic formula and procedure outlined above, a study was made of the influence of variations in formula and procedure on baking properties.

Fermentation time of starter: The starter fermentation time was varied from 6 to 11 hours in one-hour increments. The results showed that satisfactory loaves could be baked with starters fermented from 6 to 11 hours, with an optimum time of 8 to 9 hours.

Absorption: Absorption studies, summarized in Table II, showed that a shorter proofing time was required to secure optimum bread as the absorption was increased. When the optimum proof time was employed for each absorption, there was little difference in loaf volume and other bread characteristics. However, the doughs made at the highest absorptions were very mobile, sticky, and difficult to handle.

TABLE II

EFFECT OF ABSORPTION ON PROOF RATE AND LOAF CHARACTERISTICS [1]

Absorption[2]	Time of proof		Oven spring	Volume	Loaf type	Crust color	Crumb		
	To time	To height					Grain	Texture	Color
%	min	min	cm	cc					
52	150	—	1.5	535	J9	S	9	8	9
	—	175	1.5	550	J9	S	9	8	9
54	150	—	1.5	560	J10	S	9	8	9
	—	155	1.8	570	J10	S	9	8	9
56	150	—	2.0	530	J10	S	9	8	9
	—	180	1.3	535	J10	S	9	8	9
58	150	—	0.8	555	J7	S	6	9	9
	—	110	1.8	555	J10	S	6	9	9
60	150	—	0.5	555	J6	S	6	9	9
	—	125	1.5	555	J9	S	8	9	9

[1] Crumb grain, texture, and color judged on the basis of 10 as a perfect score. S = satisfactory.
[2] Absorption is expressed on the basis of the combined weight of flour and milk solids (15% moisture basis) in the dough.

Effect of milk: Kohman (1912) found that starters cultured without milk developed more slowly but gassed for a longer period; the increase in acidity of such cultures was less than when milk was included. Baking tests were accordingly conducted in which increments of skim-milk powder were added to the starter only, and to sponges and doughs, prepared with starter, containing the minimum quantity of milk that would provide for its adequate development in about 12 hours. Typical results are shown in Table III.

Starters cultured without milk were very slow in developing and required approximately 16 hours of fermentation before they were ready for making up the sponges. Sponges prepared from milk-free starters were also slow, taking from 5 to 6 hours to reach optimum development. However, the doughs made entirely without milk proofed as rapidly as did those containing it. Significantly, the milk-free loaves were completely lacking in the cheeselike odor and taste of salt-rising bread.

Milk added at one stage either to starters or to sponges and doughs made up from minimal milk starters gave loaves inferior to those obtained by the basic formula in which milk was added to both the starter and the dough. Great tolerance to wide variations of milk

TABLE III

EFFECT OF MILK SOLIDS ON SPONGE TIME, PROOF RATE, AND LOAF CHARACTERISTICS

Milk added[1]				Sponge time	Proof time		Oven spring	Loaf volume	Loaf type	Crust color[2]	Crumb		
Starter	Sponge	Dough	Total		To time	To height					Grain	Texture	Color
%	%	%	%	min	min	min	cm	cc	(10)		(10)	(10)	(10)
0.0	—	0.0	0.0	330	—	140	1.0	535	J10	P	9	10	10
BASIC FORMULA													
1.6	—	1.2	2.8	150	150	—	1.3	550	J10	S	9	9	8
MILK ADDED TO STARTER ONLY													
0.48	—	—	0.48	240	120	—	3.0	535	I8	P	10	9	10
0.48	—	—	0.48	240	—	180	1.0	545	J9	P	8	7	9
1.44	—	—	1.44	170	120	—	3.6	495	H7	Sl P	10	9	10
1.44	—	—	1.44	170	—	315	1.0	555	J8	S	7	7	7
2.40	—	—	2.40	180	120	—	3.6	480	H7	Sl D	10	9	10
2.40	—	—	2.40	180	—	305	1.0	545	J8	Sl D	8	7	8
3.35	—	—	3.35	150	120	—	3.8	490	H7	D	10	9	10
3.35	—	—	3.35	150	—	230	1.3	545	J8	D	8	7	8
4.80	—	—	4.80	125	120	—	3.8	515	H8	D	10	9	10
4.80	—	—	4.80	125	—	235	1.3	550	J9	VD	8	7	8
MILK ADDED TO STARTER AND SPONGE													
0.48	1.15	—	1.63	190	150	—	4.3	460	H6	Sl P	9	8	10
0.48	1.15	—	1.63	190	—	250	1.8	480	J8	D	8	8	8
0.48	2.32	—	2.80	165	150	—	4.3	515	H8	S	9	8	10
0.48	2.32	—	2.80	165	—	450	0.5	490	J6	D	8	7	7
0.48	3.42	—	3.90	165	Dough failed to ferment								
MILK ADDED TO STARTER AND DOUGH													
0.48	—	1.15	1.63	140	150	—	4.6	460	H6	Sl P	9	9	10
0.48	—	1.15	1.63	140	—	195	1.3	525	J9	S	10	9	9
0.48	—	2.32	2.80	195	150	—	4.6	465	H6	S	9	9	10
0.48	—	2.32	2.80	195	—	255	2.0	480	J6	D	7	7	7
0.48	—	3.42	3.90	255	150	—	2.3	505	J9	D	9	10	9
0.48	—	3.42	3.90	255	—	410	1.0	505	J7	D	7	7	7

[1] The milk percentages given for the starter and sponge are expressed on the total flour and milk solids on a 15% moisture basis in the finished dough.
[2] D = dark; P = pale; S = satisfactory; Sl = slightly; V = very.

content was shown when milk was added at the starter stage. Thus doughs with 0.048% and 4.8% milk solids (total flour and milk basis) added to the starter showed little difference in proofing time or loaf characteristics except for a darkening of the crust color with increasing milk content. However, when milk was added at the later stages of fermentation, particularly to the dough, progressive inhibition of fermentation was manifested with increasing milk content.

Effect of sucrose: The results of baking tests in which sucrose was added to sponges and doughs in quantities varying from 0% to 6% (total flour and milk solids basis) in 2% increments are shown in Table IV. Sucrose had little effect on dough proof time or loaf properties when used in the sponge but appeared to retard the fermentation when added at the dough stage. Thus the doughs to which 6% sucrose was added could not be proofed to height even after 4 hours, and those baked after only 2.5 hours of proof gave typically underfermented loaves.

TABLE IV

EFFECT OF SUCROSE ON PROOF RATE AND LOAF CHARACTERISTICS

Sucrose dosage[1]	Proof time		Oven spring	Loaf volume	Loaf type	Crust[2] color	Crumb		
	To time	To height					Grain	Texture	Color
%	*min*	*min*	*cm*	*cc*					
0	150	—	0.3	550	J4	P	4	7	8
		95	2.0	545	J9	VP	10	9	9
SUCROSE ADDED TO SPONGE									
2	150	—	1.0	530	J7	P	6	8	8
	—	105	1.3	545	J9	P	10	9	9
4	150	—	0.0	530	J5	S-P	5	7	8
	—	95	1.5	575	J8	S-P	9	9	9
6	150	—	1.0	525	J7	S	7	8	8
	—	100	1.5	550	J8	S	9	9	9
SUCROSE ADDED TO DOUGH									
2	150	—	1.5	535	J10	S	10	9	9
	—	150	1.5	535	J10	S	10	9	9
4	150	—	1.5	440	H7	D	8	8	10
	—	240[3]	3.3	480	J8	D	8	8	9
6	150	—	4.8	480	H7	VD	8	9	10
.	—	240[3]	2.5	490	J6	VD	9	8	9

[1] Sucrose percentages given are based on the combined weights of flour and milk solids (both containing 15% moisture) in the dough.
[2] See footnote 2, Table III.
[3] These doughs did not proof to height by this time.

Effect of potassium bromate: The results of experimental baking tests, in which nil, 0.001%, 0.003%, 0.007%, and 0.009% potassium bromate (calculated on the basis of the total flour and milk solids in the dough) were superimposed on the basic formula at the sponge stage, are recorded in Table V. This reagent markedly checked the characteristic tendency of salt-rising doughs to slacken with fermentation. The improvement in stability with increasing increments of bromate was accompanied by progressive decreases in loaf volume

when the doughs were proofed for a fixed time; moreover, the times required to proof to height were greatly increased. These observations strongly indicate that the bromate depressed both the proteolytic and gassing functions of the bacteria.

TABLE V

Effect of Potassium Bromate on Proof Rate and Loaf Characteristics

$KBrO_3$[1]	Proof time		Oven spring	Loaf volume	Loaf type	Crust[2] color	Crumb		
	To time	To height					Grain	Texture	Color
	min	*min*	*cm*	*cc*					
0.0	150	—	1.5	600	J8	S	8	8	9
	—	110	1.5	550	J10	S	9	9	9
1.0	150	—	3.3	575	G9	S	10	10	10
	—	140	2.3	600	G9	S	9	9	9
3.0	150	—	3.3	480	H8	P	9	9	10
	—	345[3]	3.3	610	G10	S	8	9	8
7.0	150	—	3.0	390	H5	VP	7	8	8
	—	330[3]	3.6	495	G7	P	7	9	7
9.0	150	—	3.3	375	H5	VP	6	7	8
	—	340[3]	3.8	405	H7	P	7	7	7

[1] Dosage of potassium bromate is given as mg per 100 g flour and milk solids (each containing 15% moisture).
[2] See footnote 2, Table III.
[3] Doughs did not proof to height in this time.

The inclusion of bromate in the sponge was reflected in increased oven spring and change in loaf symmetry from the J type with a small break to the G type with a bold top and large break.

Biochemical Properties

The results of the baking tests suggest that the role of sucrose in the salt-rising fermentation is quite different from that in yeast metabolism. Moreover, the inclusion of bromate in the formula not only resulted in a decrease in proteolysis, as indicated by a lowered tendency of the doughs to slacken with fermentation, but also decreased the rate of gas production, as reflected in the longer times required for the bromated doughs to proof to height. These observations made it of interest to conduct comparative tests of factors influencing pH, the rate of gas production, changes in sugar level, and the extent of proteolysis in salt-rising and yeast doughs.

Effect of dibasic sodium phosphate on pH: Changes in pH during the fermentation of starters, sponges, and doughs, prepared according to the basic method, were followed by means of a glass electrode. Parallel tests were conducted on a sponge made without the addition of phosphate and also on a dough prepared therefrom (Table VI).

In the starter the acidity increased markedly between the third and sixth hours, indicating rapid bacterial growth during this period. The buffering effect of dibasic sodium phosphate in the sponge is clearly shown and the higher pH's resulting from its use are evident

TABLE VI

EFFECT OF FERMENTATION ON pH OF STARTER, SPONGES, AND DOUGHS

Fermentation time	Starter	Sponge		Dough[1]	
		Without phosphate buffer	With phosphate buffer	Without phosphate buffer	With phosphate buffer
hrs	*pH*	*pH*	*pH*	*pH*	*pH*
0	—	6.3	7.5	5.6	6.3
1	7.8	5.8	7.2	5.5	6.1
2	7.6	5.4	6.8	5.4	6.0
3	7.3	5.1	6.3	5.3	5.8
4	6.9	5.0	5.8	5.3	5.8
5	6.5	5.0	5.6	—	—
6	6.1	5.0	5.6	—	—
7	5.8	—	—	—	—
8	5.7	—	—	—	—
9	5.5	—	—	—	—
10	5.4	—	—	—	—
11	5.4	—	—	—	—
24	5.1	4.8	5.1	4.9	5.2

[1] Doughs were prepared from sponges made with and without phosphate buffer which were fermented for 2½ hours.

in the dough. It is notable that the increased pH in the dough resulting from the presence of phosphate is only in the order of 0.5 at the third hour—that is, at the completion of the normal proofing period. The higher pH, however, is reflected in marked improvement in bread quality.

Gas-production studies: Gas-production tests were carried out in duplicate at 38°C by the pressuremeter method, with 78 g of starter prepared in the regular manner outlined in the baking test procedure. The mean cumulative manometric readings at hourly intervals for the period 0–12 hours are shown in Figure 1. The quarter-hourly and half-hourly rates calculated from the data are plotted in Figure 2. In view of the large volume of gas produced, it became necessary to release the pressure several times. It will be noted that even relatively low pressures markedly decreased the rate of gas production, as indicated particularly by the increased rates following release of the gas. Similar effects of pressure were noted in gas-production studies with yeast sponges when the pressure exceeded about 100 mm of mercury. These results are not in accord with the observations of Sandstedt and Blish (1936) on yeast doughs, who found that the rate

of fermentation was not significantly influenced by changes in pressure over a wide range.

Salt-rising sponges were prepared with and without dibasic sodium phosphate in 9-hour starters, according to the formula given under the baking test procedure. Thirty-gram portions of the sponges were

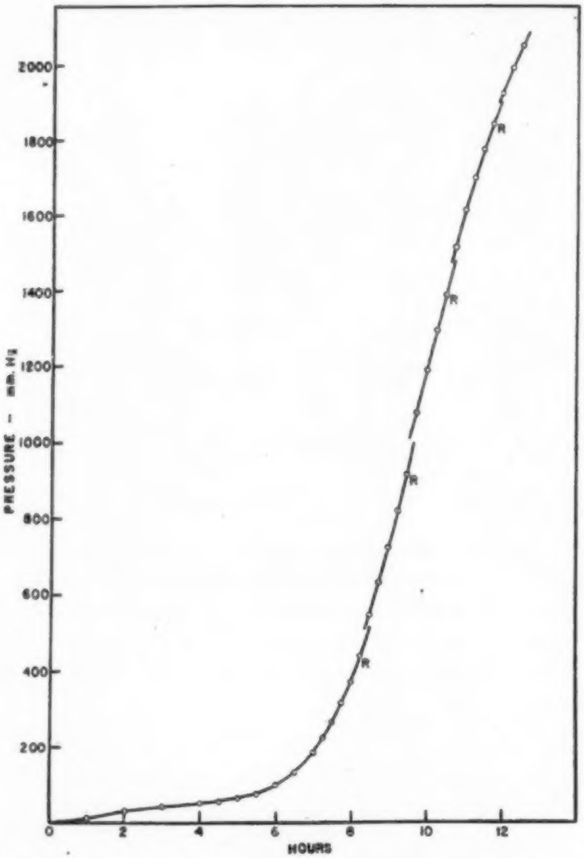

Fig. 1. Total gas production by salt-rising starter after varying fermentation times showing the effect of pressure.

made up in duplicate in 100-ml beakers, which were then placed in the pressuremeters. Zero time was taken 10 minutes after immersion of the pressuremeters in the constant-temperature bath (38°C). The pronounced stimulating effect of phosphate on the fermentation is shown by the mean gas-production curves in Figure 3. At the end of eight hours, the phosphated sponge gave a pressure of 623 mm of mercury, as contrasted with 341 mm for the nonphosphated sponge. Rate curves computed from the data showed that the addition of

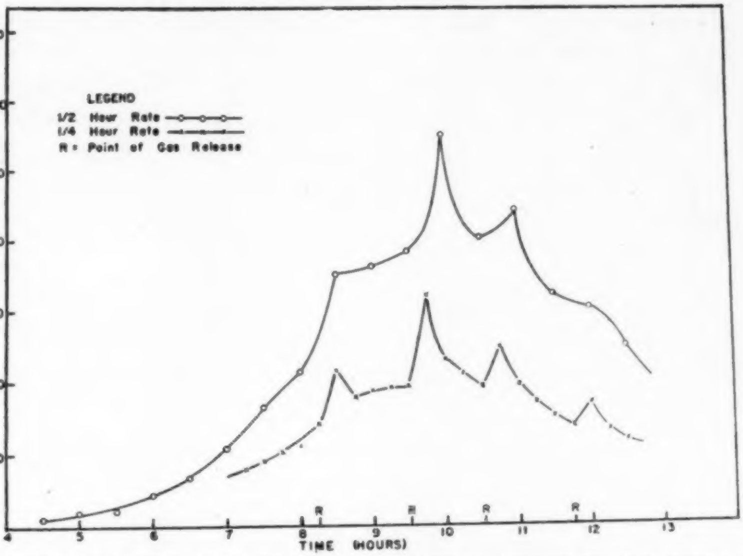

Fig. 2. Rates of gas production by salt-rising starter at various fermentation times showing the effect of releasing gas pressure.

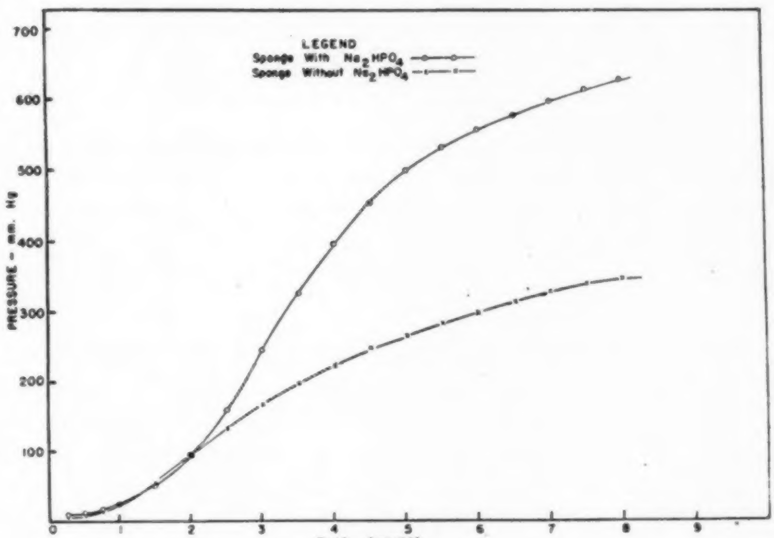

Fig. 3. Effect of dibasic sodium phosphate on gas production in salt-rising doughs.

phosphate shifted the maximum rate of gas production from the second to the third hour of fermentation.

The effect of potassium bromate on gas production was determined with sponges containing nil, 0.007%, and 0.014% potassium bromate, respectively. These bromate levels are expressed on the basis of the

flour and milk solids present in the sponge and are equivalent to 0.004% and 0.008% when expressed on the total flour and milk solids in the dough. The mean gas production curves for duplicate determinations on 30-gram sponges are shown in Figure 4. These show

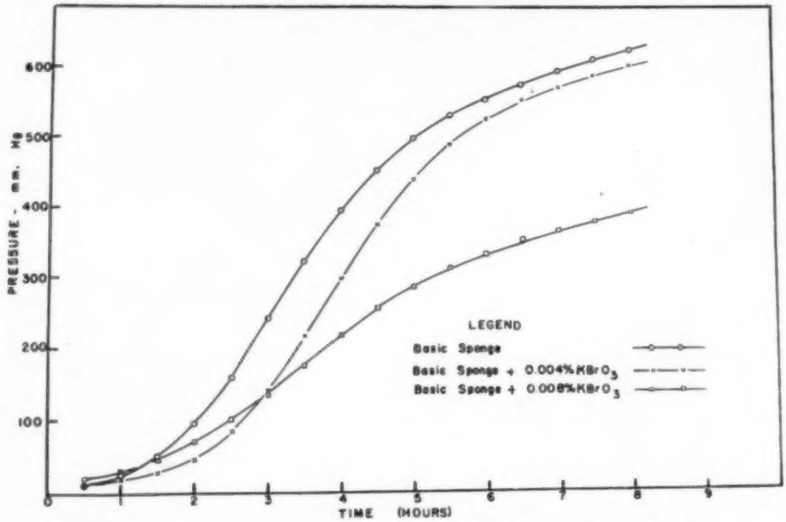

Fig. 4. Effect of potassium bromate on gas production in salt-rising sponges. The bromate concentrations shown are based on the total flour and milk solids used in making the dough.

that the lower bromate dosage had a slight retarding effect on gas production, while the higher dosage had a pronounced inhibitory effect. A study of the gas production rates revealed that the bromated sponges exhibited maximum rates at about 4 hours of fermentation as compared with 3 hours for the nonbromated sponge.

TABLE VII

Effect of Dibasic Sodium Phosphate on Gas Production in Salt-Rising Doughs

Fermentation time	Mercury pressure	
	Without phosphate	With phosphate
hrs	*mm*	*mm*
0.5	30	32
1.0	45	56
1.5	59	81
2.0	68	106
2.5	76	129
3.0	82	151
3.5	86	170
4.0	90	187
4.5	94	200
5.0	97	213
5.5	101	224
6.0	103	235

Gas-production tests were made with 40-g portions of salt-rising doughs prepared as outlined in the baking procedure and also with the omission of the phosphate. The mean results of duplicate determinations (Table VII) show a marked increase due to phosphate and serve to explain its beneficial effect on baking properties.

In order to compare rates of gas production in salt-rising and yeast doughs, a 3% yeast dough (containing the same proportions of flour, salt, sugar, and milk solids) was prepared by a sponge and dough method analogous to that employed in making the basic salt-rising dough. Forty-gram portions were fermented in the pressuremeters at 30°C. The mean results of duplicate determinations of gas production, recorded in Table VIII, together with data for the same

TABLE VIII

Relative Gas Production in Salt-Rising and Yeast Doughs [1]

Time	Mercury pressure	
	Basic salt-rising dough	3% yeast dough
hrs	*mm*	*mm*
0.5	32	121
1.0	56	252
1.5	81	371
2.0	106	482
2.5	129	591
3.0	151	668
4.0	187	778
5.0	213	825

[1] Salt-rising dough fermented at 38°C; yeast dough at 30°C.

quantity of salt-rising dough fermented at 38°C, show that the yeast doughs produced more gas than salt-rising doughs of similar composition.

Reducing sugar levels in salt-rising and yeast doughs: Kohman (1912) observed that the addition of lactose to a casein culture of salt-rising bacteria did not stimulate gas production. In the present studies sugarless salt-rising sponges and doughs fermented and proofed almost as rapidly as those containing added sucrose. These findings indicate that sugar is not involved in the production of gas by the bacteria. Accordingly a comparative study was made of the reducing-sugar levels in salt-rising and yeast doughs made without added sucrose. The following doughs were prepared at 55% absorption:

1. Sugarless salt-rising dough. The basic formula was used except that sugar was omitted.
2. No-starter, sugarless, salt-rising dough. The dough contained the ingredients of the preceding dough but was made up from a sponge to which no starter or dibasic sodium phosphate had been added.

3. Sugarless straight yeast dough. A 3% straight yeast dough made without sucrose, but containing the same proportions of salt, milk solids, and shortening as the salt-rising doughs.

4. Sugarless yeast dough, sponge method. This dough was made by the same formula as the straight yeast dough but 60% of the flour, 50% of the milk solids, and all the water and yeast were sponged and fermented at 30°C for three hours prior to making up the dough.

The salt-rising doughs were kept at 38°C and the yeast doughs at 30°C; reducing-sugar determinations were made at intervals of 0, 1, 2, 3, and 4 hours by the method previously outlined. The results, presented graphically in Figure 5, indicate little or no sugar consump-

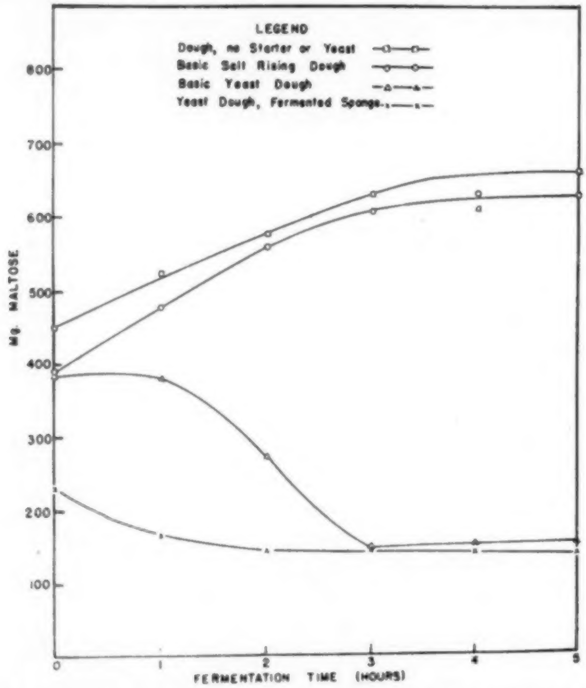

Fig. 5. Changes in reducing sugar content of salt-rising and yeast doughs with fermentation. The maltose content is expressed as milligrams of maltose per dough weight equivalent to 100 g flour and milk solids (15% moisture basis).

tion by the bacteria. The reducing sugar contents of the nonfermenting and fermenting salt-rising doughs were nearly equal and increased with time as a result of amylase activity. In striking contrast, yeast fermentation rapidly lowered the reducing-sugar content of the yeast doughs to a low level.

Titratable acidity and proteolytic activity: The formol titration procedure furnished an index of both the titratable acidity and amino nitrogen content of sponges and doughs at various stages of fermentation.

Acidity determinations on salt-rising sponges prepared in the regular manner, with the omission of phosphate, starter, and milk solids, and with the addition of 0.004% potassium bromate (expressed on the basis of total flour and milk solids in dough), are shown graphically in Figure 6. In the control sponge, made without starter but con-

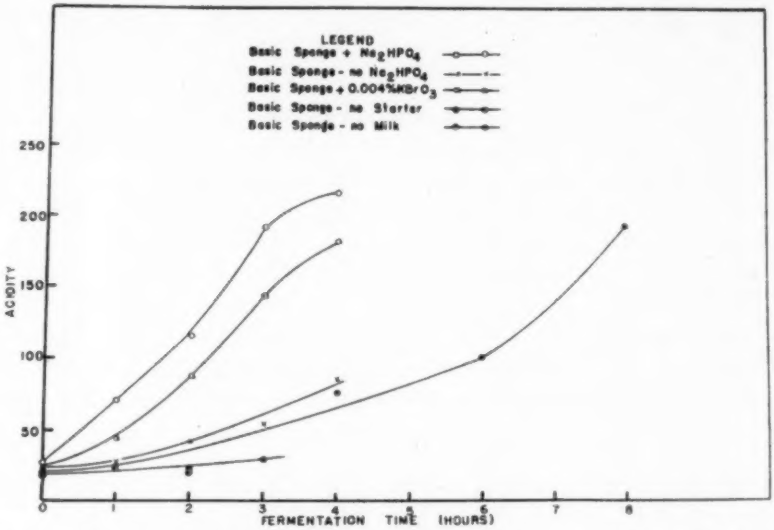

Fig. 6. The development of titratable acidity in salt-rising sponges showing the effect of sodium phosphate, potassium bromate, and milk solids. Titratable acidity is expressed as ml $N/14$ NaOH/100 g flour and milk solids in the sponge.

taining all other ingredients including phosphate, the titratable acidity increased only very slightly with time. The highest development of acidity was given by the basic sponge and the omission of phosphate resulted in a very marked decrease. Bromate slightly depressed the acidity. The curve for the sponge made from starter in which milk was omitted is in line with the very long period required for the development of sponges noted in the baking studies.

Titratable acidity was followed in a basic salt-rising dough prepared in the regular manner, in a basic dough plus 0.004% potassium bromate (total flour and milk solids basis, bromate added to sponge), and in a dough made without the use of starter in the sponge. A comparison of the results given in Table IX with those obtained for sponges (Fig. 6) shows that the differences in titratable acidity of sponges due to bromate are considerably reduced in the dough.

The titratable acidity values presented above represent the first stage of the formol titration, and hence amino nitrogen values were obtained for the same sponges and doughs. The mean results for the

TABLE IX

Titrable Acidity of Salt-Rising Doughs

Fermentation time	*N*/14 NaOH per 100 g flour and milk solids		
	Basic dough less starter	Basic dough	Basic dough $+0.004\%$ $KBrO_3$
hrs	*ml*	*ml*	*ml*
0	19.5	115.5	110.0
1	22.4	131.0	123.0
2	26.1	146.0	134.5
3	27.8	162.0	149.5
4	31.4	175.0	163.0
	AMINO NITROGEN PER 100 G FLOUR AND MILK SOLIDS		
hrs	*mg*	*mg*	*mg*
0	30.7	51.6	49.2
1	31.7	62.3	59.4
2	32.2	73.6	68.7
3	32.4	80.4	76.0
4	28.8	86.0	82.6
5	30.0	93.5	87.5

sponges, shown graphically in Figure 7, reveal that proteolysis is most extensive in the basic sponge and is markedly inhibited by bromate. In the absence of phosphate, proteolysis proceeded initially at a more

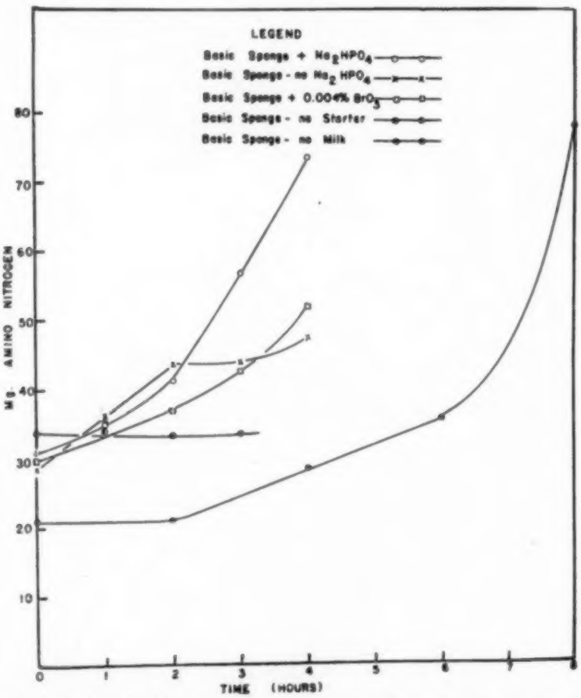

Fig. 7. Proteolysis in salt-rising sponges showing the effect of sodium phosphate, potassium bromate, and milk solids. Amino nitrogen content is expressed as mg per 100 g flour and milk solids in the sponge.

rapid rate than in the sponge containing the buffer salt but fell off sharply after two hours. This decrease in rate is doubtless associated with the general loss in fermentative activity noted in the previous tests. Similarly the low proteolytic activity in the milk-free sponges is correlated with the low rate of increase in titratable acidity and the slow sponge development observed in the baking tests when no milk was used.

The data in Table IX reveal a slight increase in amino nitrogen content of doughs made from sponges containing no starter (due probably to the inherent flour proteases) up to the third hour; the subsequent slight drop may be attributed to the activity of wild yeasts. With regard to the influence of bromate, the initial level of amino nitrogen was lower in the bromated dough as a result of the inhibition of proteolysis during the sponge stage where the bromate was added. In the bromated and nonbromated doughs, however, the rates of amino nitrogen production were virtually identical. A comparison of these data with those obtained by Shen and Geddes (1942) for yeast doughs in which utilization of the amino nitrogen by the yeast was prevented by the addition of octyl alcohol, shows that proteolysis in salt-rising doughs is much greater.

Effects of Salt-Rising and Yeast Fermentation on Physical Properties of Doughs

As a measure of the relative effects of salt-rising and yeast fermentation on the physical properties of doughs, a series of tests was made with the Brabender farinograph and extensograph.

For the farinograph tests, a basic salt-rising dough, a similar dough containing 0.004% potassium bromate (added at the sponge stage), and a nonbromated straight-yeast dough made by a formula containing 3% yeast and the same percentages of sugar, salt, shortening, and milk as the salt-rising doughs, were used. All three doughs were mixed to 55% absorption, divided into 80-g aliquots, rounded up and fermented in covered 300-ml beakers for varying times. The salt-rising doughs were fermented at 38°C, the yeast doughs at 30°C and farinograms were made in the small mixer at 30°C. The consistencies recorded after 10 minutes of mixing are given in Table X. While the Brabender consistency values of all three doughs markedly decreased with fermentation, the relative values were in striking contrast to the observed consistency as reflected in handling properties. The zero time consistency of the yeast dough was 20 units less than for the salt-rising doughs, and the consistency decreased at a more rapid rate than did that of both salt-rising doughs. Yet, at zero time, 60% of the flour used in the salt-rising doughs had already undergone 150

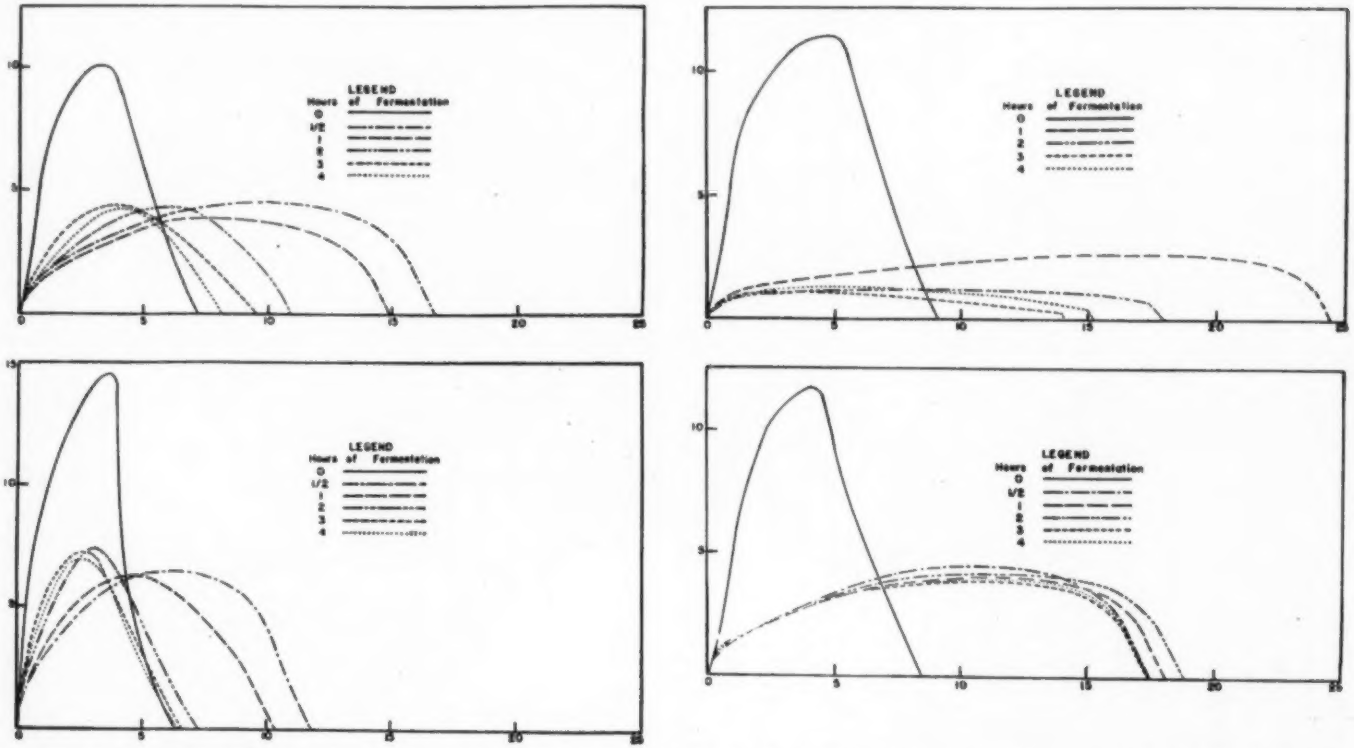

Fig. 8. Extensograms for bromated and nonbromated salt-rising and yeast doughs after different fermentation times. Extensograms for yeast doughs are shown in the lefthand column and for salt-rising doughs in the righthand column. Those in the top row are for nonbromated doughs and those in the bottom row are for bromated doughs. The graphs were made from direct tracings of the extensograph curves.

minutes of fermentation in the sponge and these doughs were noticeably of somewhat lower consistency than the yeast dough. At the 5-hour fermentation time the nonbromated salt-rising dough could be poured from the beaker as a result of extensive proteolysis, but it exhibited a higher Brabender consistency by 80 units than the yeast

TABLE X
EFFECT OF SALT-RISING AND YEAST FERMENTATION ON BRABENDER CONSISTENCY VALUES

Fermentation time	Salt-rising dough: Without bromate	Salt-rising dough: With 0.004% bromate	Yeast dough
hrs	*Brabender consistency units*		
0	500	500	480
1	470	480	440
2	450	460	420
3	440	430	380
4	430	420	360
5	420	420	340

dough fermented for the same time. Moreover, the bromated salt-rising doughs were observed to be much stiffer than the nonbromated doughs, particularly as the fermentation time was extended. It would appear that the farinograph fails to interpret the great decrease in consistency of the salt-rising doughs with fermentation because of their increased stickiness.

Extensograms were made with salt-rising and yeast doughs prepared with and without 0.004% potassium bromate. The formulas were the same as those employed for the farinograph tests with the exception that only 2% yeast was used in the yeast doughs; this decrease was found necessary in order to secure better replication in the extensograph. In the instance of salt-rising doughs, six 150-gram aliquots taken from a large mix were scaled off, rounded up, molded with the auxiliary extensograph equipment, and placed in the dough holders. An extensogram was made with one aliquot immediately after mixing while the others were reserved for tests after fermenting at 38°C for various times. In the instance of the yeast doughs, the aliquots were fermented in covered beakers at 30°C until 30 minutes before the end of the fermentation period. At this time the doughs were rounded, molded, and clamped in the dough holders. Extensograms were made after fermenting in the holders for 30 minutes at 30°C. Photographs of direct tracings of the extensograms are reproduced in Figure 8. Munz and Brabender (1940) have shown that differences in the physical properties of doughs as a result of various treatments are best reflected by the extensograph when studied after a period of rest. When tested immediately after mixing, the "excited

state" of the doughs greatly influences the character of the extensogram. This observation is fully confirmed here. The extensograms graphically illustrate the marked change in extensibility of the salt-rising doughs with fermentation and the increase in stability resulting from the inclusion of potassium bromate in the formula. A notable feature of these curves is the narrow range of the vertical component (stress) and the great variability in the horizontal component (strain) with increasing fermentation time. Both the nonbromated and bro-

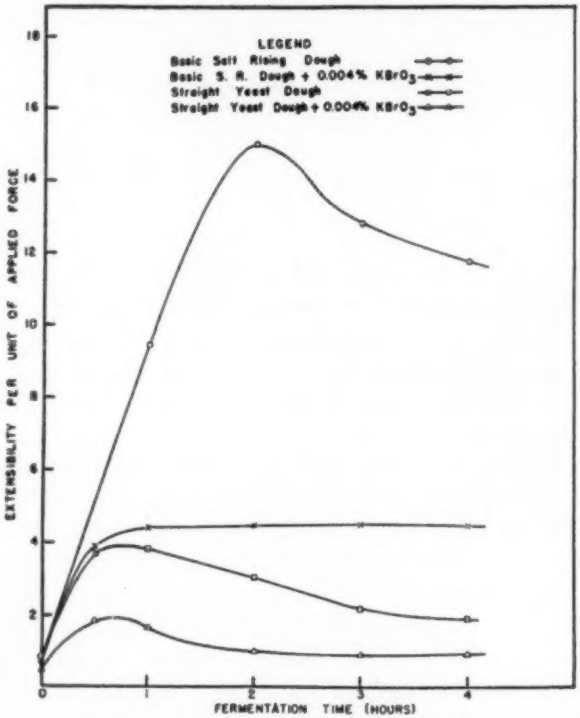

Fig. 9. Changes in extensibility/force ratios for bromated and nonbromated salt-rising and yeast doughs with fermentation.

mated yeast doughs exhibit a much lower extensibility than the salt-rising doughs at the first hour of fermentation and a gradual "tightening" or decrease in extensibility upon further fermentation. The "tightening" effect of bromate, noted for the salt-rising doughs, is also evident with the yeast doughs. The relative changes in the physical properties of the salt-rising and yeast doughs with fermentation and the effect of bromate may be clearly shown by computing the strain values per unit stress or, using the terminology of Munz and Brabender, the "extensibility (E) per unit of maximum force (F)." The E/F ratios are shown in Figure 9.

Discussion

The marked differences in physical and biochemical properties between salt-rising and yeast doughs can be ascribed to the distinctive metabolism of the fermentative organisms involved. Salt-rising fermentation is marked by a high degree of proteolysis which results in doughs and loaves possessing unique properties. The structure and properties of such doughs and loaves are highly sensitive to overproof, the doughs exhibiting a high rate of production and increasing content of amino nitrogen with fermentation.

Buchanan and Fulmer (1930) make this statement: "The fact that bacterial proteases are inhibited by acids and work best in neutral or alkaline solutions has led to their being classified with the tryptases by many authors." If it is assumed that the proteolytic action of salt-rising fermentation can be ascribed to the secretion of such tryptic type enzymes by the bacteria involved, the beneficial effects obtained by the inclusion of the basic buffering salt on the fermentative activity may be accounted for. The higher pH levels (5.5–7.5) required for optimum fermentative activity appeared necessary not only for the proteolytic function but for the gas-producing function as well. The organisms were found to utilize little if any reducing sugars in their gas-producing function, which is apparently connected with protein decomposition and utilization.

Proteolytic micro-organisms produce a large variety of organic acids as well as intermediate protein decomposition products. Anaerobic spore formers, into which class salt-rising organisms probably fall, are known to produce a variety of amines by their action on casein. The characteristic odor and taste of salt-rising bread, when it includes milk solids in its makeup, may accordingly be accounted for. In these studies, salt-rising loaves prepared without milk solids completely lacked the characteristic odor.

Potassium bromate in relatively high concentrations markedly inhibited proteolysis in the sponge. As gas production and titratable acidity were also lower, the bromate appeared to retard the metabolic activity of the organism as a whole. At the dough stage, in which the bromate concentrations were lower than in the sponge, the rate of increase in amino nitrogen was virtually identical for the bromated and nonbromated doughs. The dough-handling properties and stability to fermentation were markedly superior when bromate was added at the sponge stage. The regular doughs became slack and runny after 2 hours' fermentation, yielding flat-topped loaves with coarse open grain. Similar doughs containing 0.001% to 0.003% potassium bromate (total flour and milk solids basis) added to the sponge re-

mained stable up to $5\frac{3}{4}$ hours of fermentation and yielded loaves of large volume for this type of bread. These large volumes were obtained in spite of the lower gas production and indicate increased gas retention in the bromated doughs.

It is of interest to note that the farinograph failed to record the pronounced slackening of the salt-rising doughs or the "tightening effect" of potassium bromate. In fact the farinograph indicated that salt-rising doughs, after several hours of fermentation, when they would actually flow from the vessels in which they were contained, possessed a greater consistency than yeast doughs which were much firmer and of higher stability at similar fermentation periods. This anomaly is probably due to the increasing stickiness of the salt-rising doughs with fermentation. In striking contrast, the extensograph recorded differences in the physical properties of the salt-rising and yeast doughs which were in line with their observed physical condition. Furthermore this instrument clearly interpreted the marked stabilizing effect of bromate on dough structure and also showed that the effect of this reagent was more marked in salt-rising than in yeast doughs.

Summary

Employing a commercial preparation, known as Kohman's "salt-rising yeast," which is prepared from milk cultures of certain spore-forming bacteria present in corn meal, a study has been made of the optimum conditions for baking and the biochemical properties of the doughs.

A successful laboratory-scale procedure for the production of salt-rising bread required certain modifications of recommended commercial practice for the preparation of the starter, sponge, and dough. Satisfactory loaves were obtained by using twice the quantity of ferment recommended, by adding all the water called for by the dough formula to the sponge, and maintaining the pH at levels above 5.8 by the use of dibasic sodium phosphate as a buffering agent.

Salt-rising bread is characterized by a relatively small loaf with square corners and a smooth dark crust. The crumb is compact, of even, fine grain and smooth texture, with a characteristic cheese-like odor.

Salt-rising doughs markedly decrease in consistency with fermentation and are sensitive to overproof. Increased absorption and use of milk in the starter and sponge shortened the proof time required for optimum bread. Sucrose was without effect while potassium bromate lengthened the proof time. Starters and sponges prepared without milk fermented slowly and milk-free bread lacked the characteristic cheese-like odor and flavor of salt-rising bread.

Biochemical studies showed that little or no sugar is utilized in the production of gas by the bacteria. The fermentation is characterized by a high rate of proteolysis resulting in a marked slackening of the doughs during fermentation. Potassium bromate added at the sponge stage markedly decreased the rate of proteolysis as well as the rate of gas production and the development of acidity. Doughs prepared from bromated sponges exhibited less slackening than nonbromated doughs with fermentation.

The farinograph failed to record the marked slackening of salt-rising doughs with fermentation and the stabilizing effect of potassium bromate. On the other hand the extensograph clearly revealed the effects of fermentation and bromate as well as the markedly greater extensibility of salt-rising as compared with yeast doughs.

Acknowledgment

The authors are indebted to H. A. Kohman, Mellon Institute, Pittsburgh, for his kindness in supplying the salt-rising ferment used in these studies and a recommended formula for the commercial production of salt-rising bread.

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VISCOSITY CHANGES IN SODIUM SALICYLATE DISPERSION OF HARD RED SPRING WHEAT GLUTEN IN RELATION TO VARIETY AND ENVIRONMENT

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Harris and Johnson [2] have previously pointed out differences in the rate at which wheat glutens prepared from different classes and varieties of wheat disperse in 10% sodium salicylate solution. Samples of hard red spring, hard red winter, soft red winter, hard and soft white, and durum wheats belonging to the 1939 crop were examined, and large differences were found in dispersion rates among some classes and varieties. With three varieties of spring wheat grown at three North Dakota stations, curves obtained by plotting viscosity against time of gluten dispersion were similar with one exception. Specific volume of gluten particle (volume occupied by one gram of gluten protein) varied significantly among some of the classes, and was positively correlated with loaf volume. No relationship between these variables appeared within the hard wheat classes.

The effects of such factors as soil and weather upon these gluten properties were not included in the study mentioned above, and no data regarding varietal effects of hard red spring wheats were reported. It was therefore decided to investigate a series of glutens prepared from hard red spring wheats grown in 1939 and 1940 at various locations in North Dakota, with the object of obtaining information on these phases of the problem. Since the paper referred to summarized the literature pertaining to work upon which these researches are based, further reference to previous studies will be omitted.

Experimental Material and Methods

Sixty-eight samples of hard red spring wheat grown at six stations were obtained. These wheats consisted of 13 varieties from repre-

[1] At present, technologist, Department of Milling Industry, Kansas State College, Manhattan, Kansas.
[2] R. H. Harris and John Johnson, Jr.: A study of some differences in gluten properties existing between varieties and types of wheat, Cereal Chem. **18**: 395–413, 1941.

sentative locations within the state. Twenty were grown in 1939 and 48 in 1940. They were produced under strictly comparable conditions, and all were sound and free from damage that would tend to vitiate the results of study. Some of these varieties have been produced commercially for a number of years, while others are being examined in respect to agronomic and quality performance from year to year in comparison with the older, more "standard" varieties. The samples were cleaned and graded with standard equipment and milled into straight-grade flour on an Allis-Chalmers experimental mill. The temperature and relative humidity of the mill room approximated 70°F and 60% respectively. The flours were baked by the malt-phosphate-bromate method.

The dispersions were prepared by methods outlined in the previous report and consisted, in brief, of mixing to standard consistency by the standard basic formula with 5% sucrose. The gluten was immediately washed from the dough under a standardized stream of 0.1% sodium phosphate at pH 6.8, allowed to stand for a short period under the phosphate solution, then 10 g of the finely divided gluten was added to 100 ml of 10% sodium salicylate solution and dispersed with the aid of constant gentle agitation in an electric rotary shaker. The rate of dispersion was measured in terms of increase in viscosity by withdrawing suitable portions at two-hour intervals, centrifuging, and determining viscosity with a calibrated Ostwald pipette. The liquid and residual gluten were returned to the original flask as soon as possible after completion of the determination. The data were calculated in terms of absolute viscosity, and are presented in the form of curves in which viscosity is plotted against time of dispersion. The relative dispersion rates were also found by calculating the differential quotient dv/dt, where v is the viscosity in centipoises multiplied by 10^2, and t is the elapsed time in hours. The concentrations of protein in the centrifuged dispersions were determined at the end of 24 hours of dispersion. The specific volumes of the protein particles at this time were also computed, by the formula of Kunitz. Viscosities and dispersion rates were compared at the sixth and eighth hours and comparisons were made of viscosities of the dispersions at 6, 8, and 24 hours.

Results and Discussion

The varieties investigated cover as large a range in strength as it would be possible to find in the hard red spring wheat region. It might have been better if identical stations had been included for the two years, but Fargo and Langdon are common to both years. The wheat protein content varied from 11.6% to 17.5% and the loaf volume from 490 cc to 940 cc. There were marked differences in

viscosity at the 6th hour, but these differences were less noticeable at the 24th hour. The rates of dispersion were particularly variable, ranging from 2.59 to 17.88 after 6 hours of dispersion, whereas following 8 hours they varied from 2.27 to 15.87. These differences were much greater than those found in other parts of the study. Comparatively small differences were evident among the specific volume data.

Thatcher ranked very high in protein content, except at Fargo and Langdon in 1940. Premier, on the other hand, tended to be low in protein content and loaf volume. Their rates of gluten dispersion in sodium salicylate were also very different, Thatcher gluten being much more resistant than Premier. Regent, a comparatively new Canadian variety, also had a resistant gluten. Marquis gluten produced at Fargo and Dickinson in 1940 dispersed very slowly but dispersed more quickly when produced at Langdon. It was intermediate in dispersion rate when grown at Fargo in 1939, resembling Rival, a new variety recently released for distribution in North Dakota, and Vesta.

In Table I are shown average values by station and variety. These data are all from the 1940 crop, and comprise results obtained on 11

TABLE I

STATION AND VARIETY AVERAGES, 1940 CROP

Stations and varieties	Crude protein ($N \times 5.7$)[1]	Flour absorption[1]	Loaf volume[1]	Viscosity		Rate of dispersion		Specific volume
				6 hrs	24 hrs	6 hrs	8 hrs	
	%	%	cc	$cp \times 10^{2}$		dv/dt		ϕ/C
STATION AVERAGES								
Fargo	15.8	56.4	598	138	246	4.29	5.96	7.18
Dickinson	15.4	57.6	569	159	227	7.86	10.06	6.73
Langdon	14.8	59.9	642	186	236	12.14	11.78	7.09
Mandan	14.2	59.0	602	172	244	9.58	10.70	7.04
VARIETY AVERAGES[2]								
Thatcher	15.9	58.3	646	140	239	4.59	6.61	7.01
Pilot B	15.8	57.8	593	176	242	10.53	11.39	7.28
Rival	15.3	58.5	625	162	255	8.28	10.68	7.12
Renown	15.2	57.5	646	155	234	6.85	8.13	7.06
Pilot 13	15.2	58.4	559	156	251	7.26	9.12	7.47
Vesta	15.1	58.6	624	162	244	8.11	9.80	6.87
Merit	15.1	58.4	593	178	225	10.92	11.17	6.61
Premier	14.7	58.1	583	189	226	12.72	12.09	6.55
Marquis	14.4	58.4	599	154	241	6.72	7.74	7.09
Ceres	14.4	58.7	580	172	234	9.78	10.57	7.02
Nordhougen	14.3	57.7	585	158	241	7.42	8.64	7.06

[1] On 13.5% moisture basis.
[2] Only varieties grown at all four stations are included.

wheat varieties grown at four stations. Two other varieties, grown only at Fargo and Langdon, are not shown in the table. From station averages it is apparent that a difference of 1.6% exists in wheat protein, and a difference of 73 cc in loaf volume. The station with the highest protein content does not show the highest loaf volume, nor

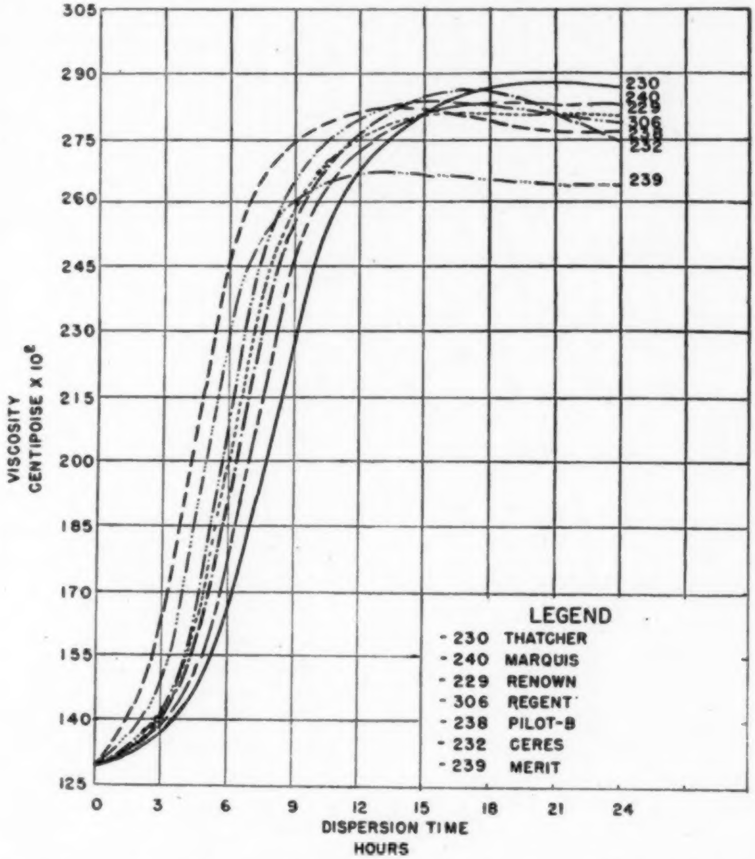

Fig. 1. Dispersion rates of glutens washed from some hard red spring wheat varieties grown at Fargo in 1939.

does the station with the lowest protein content show the lowest loaf volume. The viscosity values at six hours appear to have a tendency to increase as the wheat protein decreases, but no trend is evident after 24 hours of dispersion. Large differences in the rates of dispersion are shown, particularly at the sixth hour. These rates appear to be more or less characteristic of the station and must therefore be connected with environmental factors. The two stations with the highest average wheat protein had the lowest rates but no relation is shown

between these values for the remaining two stations. No marked differences are seen in the specific volume results.

The variety averages have a range of 1.6% in protein and 87 cc in loaf volume. Thatcher was high in both these quality characteristics, but there was little difference between this variety and several

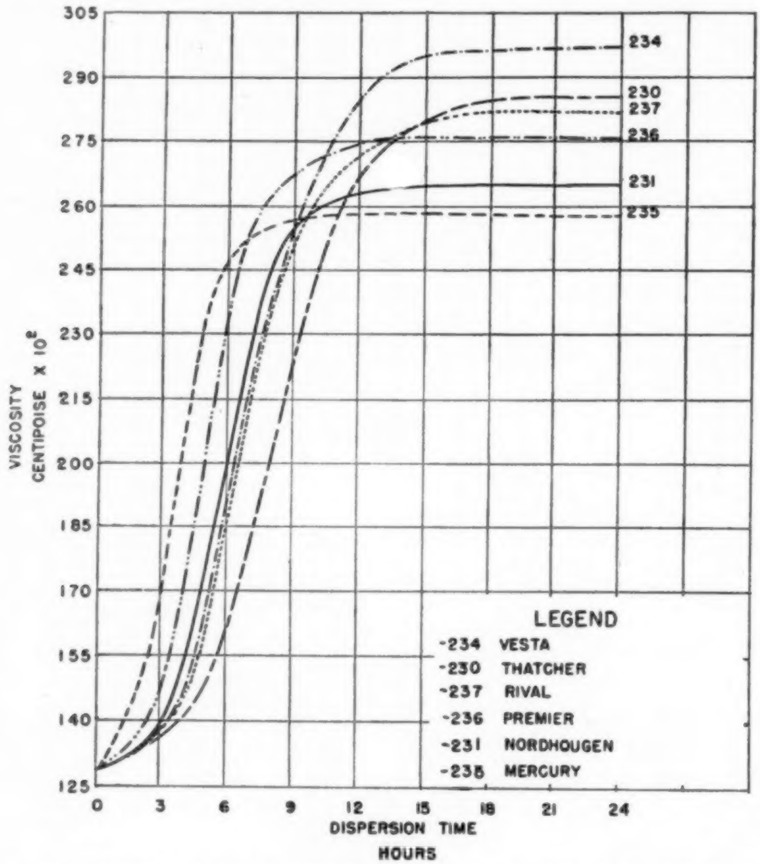

Fig. 2. Dispersion rates of glutens washed from some hard red spring wheat varieties grown at Fargo in 1939.

others. Thatcher had the lowest gluten viscosity in sodium salicylate after six hours, associated with the slowest rate of dispersion. Premier, on the other hand, had the highest gluten viscosity and dispersion rate. The other varieties are spread out between these two extremes, with Renown, Marquis, Pilot 13, and Nordhougen in the higher bracket, while Merit and Pilot B resemble Premier in viscosities and dispersion rates of their glutens. It is evident that wheat variety has a substantial influence upon the rate at which gluten disperses in 10%

sodium salicylate. These characteristic effects of variety and environment are presented graphically by viscosity plotted against time of dispersion.

Figures 1 and 2 show the dispersion rates obtained on glutens prepared from various varieties of hard red spring wheats grown at

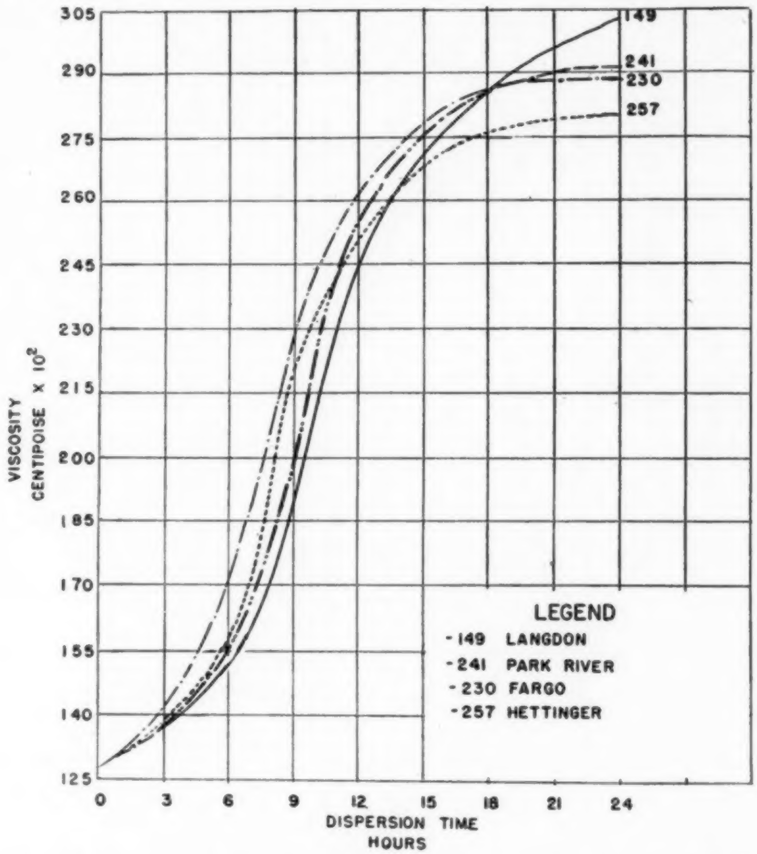

Fig. 3. Dispersion rates of glutens washed from Thatcher wheat grown at four North Dakota stations in 1939.

Fargo in 1939. It was impossible to depict the entire set of curves in one figure, and they were accordingly divided into two sets, Thatcher being included in each for purposes of comparison. In both instances the Thatcher gluten is the most resistant to dispersion. In Figure 1 Marquis follows Thatcher, while Pilot B is least resistant. Merit had the lowest viscosity after 24 hours of dispersion. Regent, Ceres, and Renown glutens have similar dispersion rates. Figure 2 shows greater differentiation than Figure 1 in final viscosities. Mercury gluten dis-

persed most rapidly, with Premier next. Rival, Nordhougen, and Vesta glutens were similar, but Vesta had the highest final viscosity.

In Figure 3 are gluten dispersion rates for Thatcher grown at four North Dakota stations in 1939, while Figure 4 shows similar data for Premier. Apparently no marked effect upon the gluten dispersion

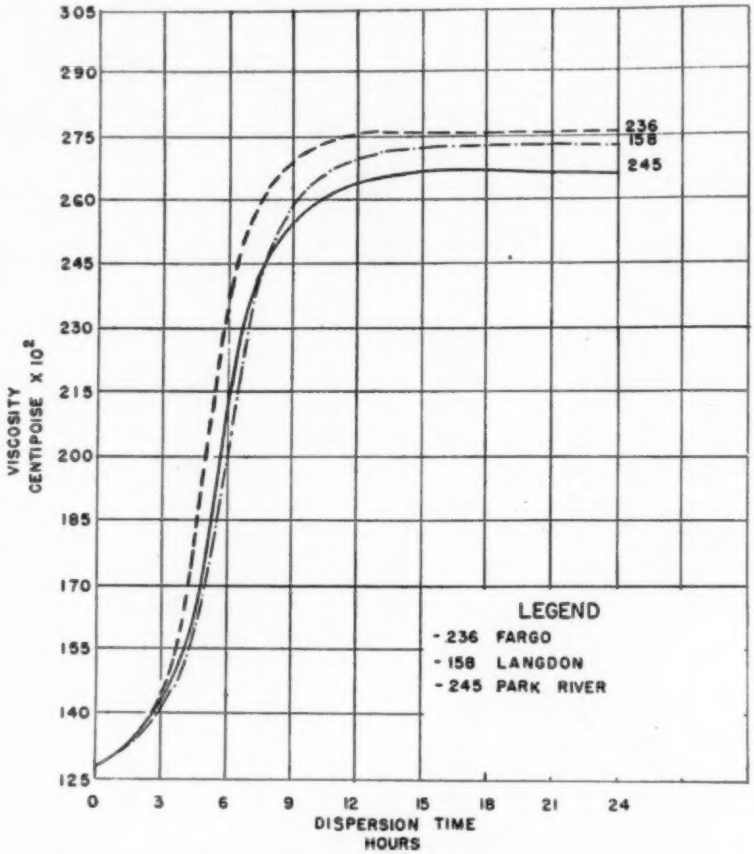

Fig. 4. Dispersion rates of glutens washed from Premier wheat grown at three North Dakota stations.

rate was exerted by environmental differences, and the curve characteristics peculiar to each variety were not greatly affected. In other words, one would conclude that variety was more influential than environment in determining rate of dispersion in 1939, but it must be borne in mind that Thatcher and Premier glutens are very different in dispersibility, and this difference in varietal and environmental effects might not hold for other varieties which are more nearly alike in their dispersion rates.

The data obtained on the 1940-crop wheat glutens are shown in Figures 5 and 6. Figure 5 presents the Fargo sample data, while Figure 6 shows results obtained at Dickinson. Thatcher was included in both figures to afford a basis of comparison. Among the Fargo wheats, Thatcher gluten was the most resistant to dispersion, while

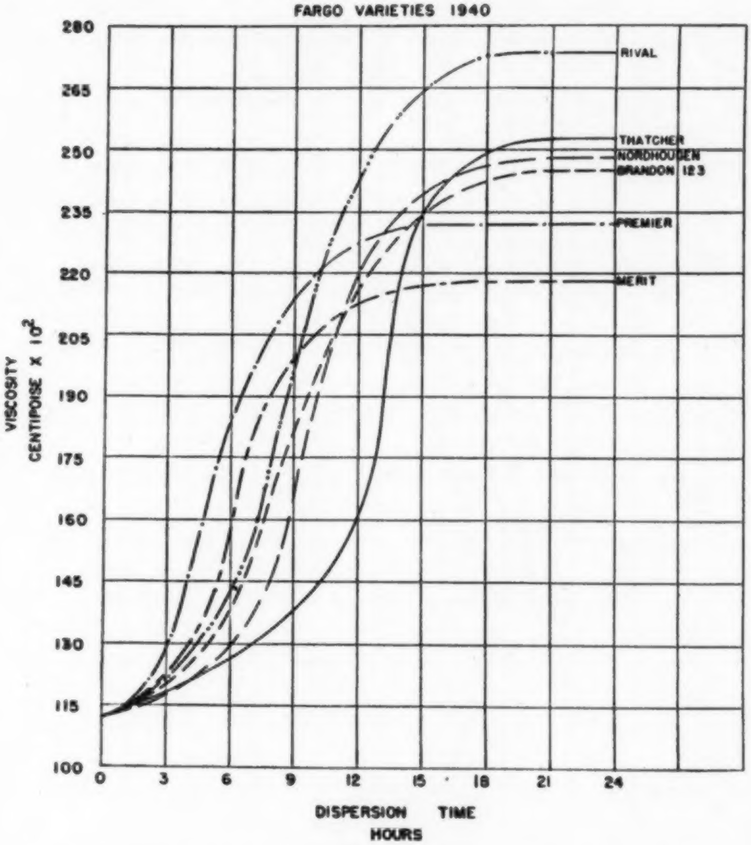

Fig. 5. Dispersion rates of glutens washed from some wheats grown at Fargo in 1940.

Premier was the least. Merit followed Premier in gluten dispersion rate and both varieties had low final viscosities in their dispersions, corresponding to the general trend shown in this figure. Rival gluten was an exception, as it had a medium dispersion rate but exhibited a high viscosity after 24 hours of dispersion. Brandon and Nordhougen glutens had dispersion rates between Thatcher and Rival. The glutens prepared from wheats grown at the Dickinson station showed less differentiation among varieties in respect to dispersion rate than the Fargo wheat glutens. Marquis was the most resistant, with

Thatcher second. Premier gluten dispersed most easily, as in the Fargo group, with Pilot B next in ease of dispersibility. Rival again had the highest final viscosity and Premier the lowest.

Figures 7 and 8 show dispersion data obtained on several varieties grown at different stations in 1940. The stations are presented in

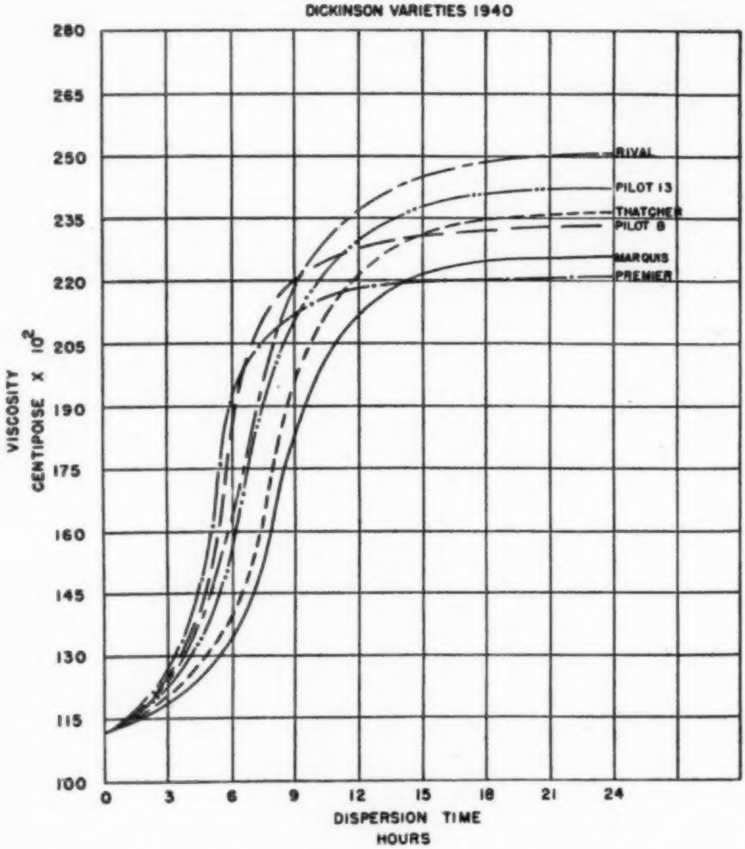

Fig. 6. Dispersion rates of glutens washed from some wheats grown at Dickinson in 1940.

groups of three. Mandan and Dickinson also gave somewhat similar curves. Figure 7 shows results from glutens prepared from Ceres and Renown grown at Fargo, Langdon, and Dickinson. These varieties were chosen because their curves were best adapted to bring out clearly the differences between stations.

Figure 7 shows distinct differences due to station effects, and emphasizes the greater changes in gluten dispersion rate caused by environmental factors than were apparent in 1939. Langdon glutens dispersed the most easily, and were very similar in 24-hour viscosity

to the Dickinson varieties. The situation depicted in Figure 8 is much the same, with Fargo again having the most resistant glutens, Mandan second, and the Langdon samples showing the least resistance for each variety except Rival at Mandan, which dispersed most rapidly of the Rival samples. The varieties arrange themselves in the follow-

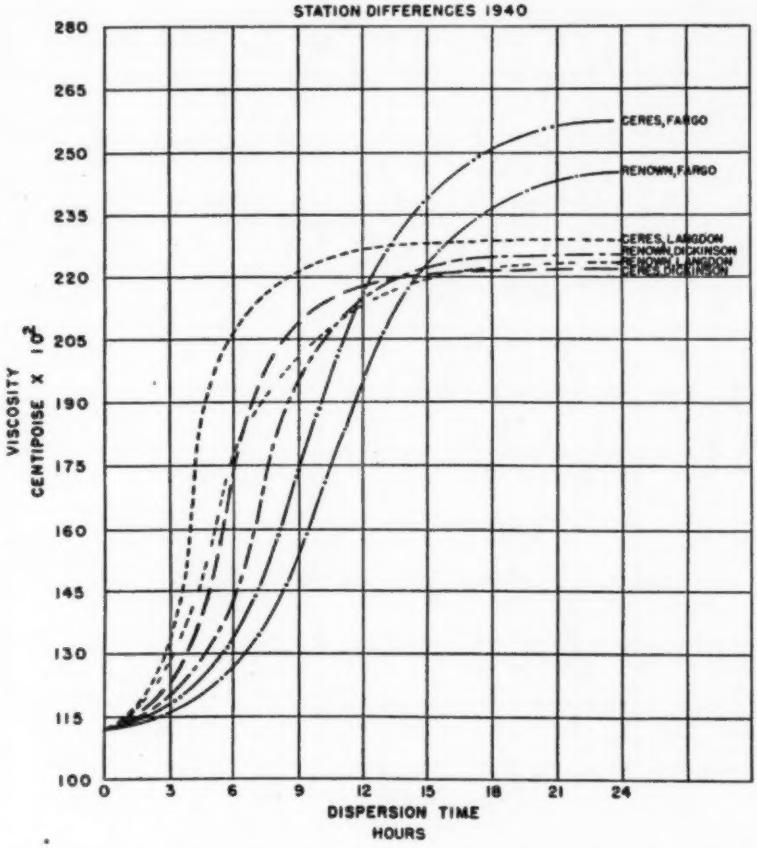

Fig. 7. Comparative gluten dispersion rates of several wheat varieties grown at different stations in 1940.

ing order of gluten dispersibility: Thatcher, Rival, Premier. Rival had the highest final viscosity for each station, while Premier had the lowest. Thatcher was intermediate in every case. The Fargo glutens had the most resistance and, excepting Mandan, Rival also had the highest final viscosity, while Dickinson was intermediate in gluten dispersion rate.

Variations in dispersibility caused by yearly differences are depicted in Figure 9. Three varieties which differed in dispersion rate

are shown. The glutens in this group were washed from wheat varieties grown at Fargo in 1939 and 1940 under comparable plot conditions, except for changes introduced by climatic variability. It is evident that the three varieties fall in the same order of dispersibility each year. It is also apparent that greater differentiation between

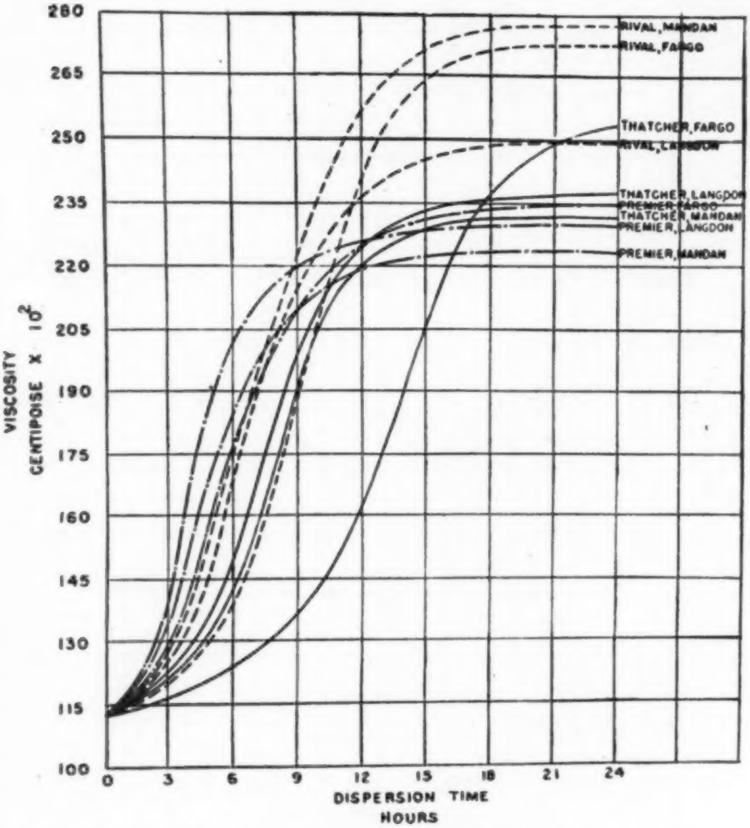

Fig. 8. Comparative gluten dispersion rates of several wheat varieties grown at different stations in 1940.

varieties existed in 1940 than in 1939, while the 24-hour viscosity was slightly higher in 1939. The 1940 glutens tended to be more resistant to dispersion than the corresponding 1939 glutens. These differences were no doubt caused by seasonal variations, and probably are closely related to station differences in these properties.

The results lead to the conclusion that variety has a distinct effect upon the rapidity or ease of dispersion of wheat gluten in 10% sodium salicylate. This varietal effect was shown at different stations where the wheats were grown, and for different crop years. There was also

a marked effect due to station, with Fargo producing the most resistant glutens and Langdon the least resistant, while Mandan and Dickinson were intermediate. These differences appear to vary in magnitude from year to year, and might even be reversed. Wheats grown in different crop years have different dispersion rates, but the

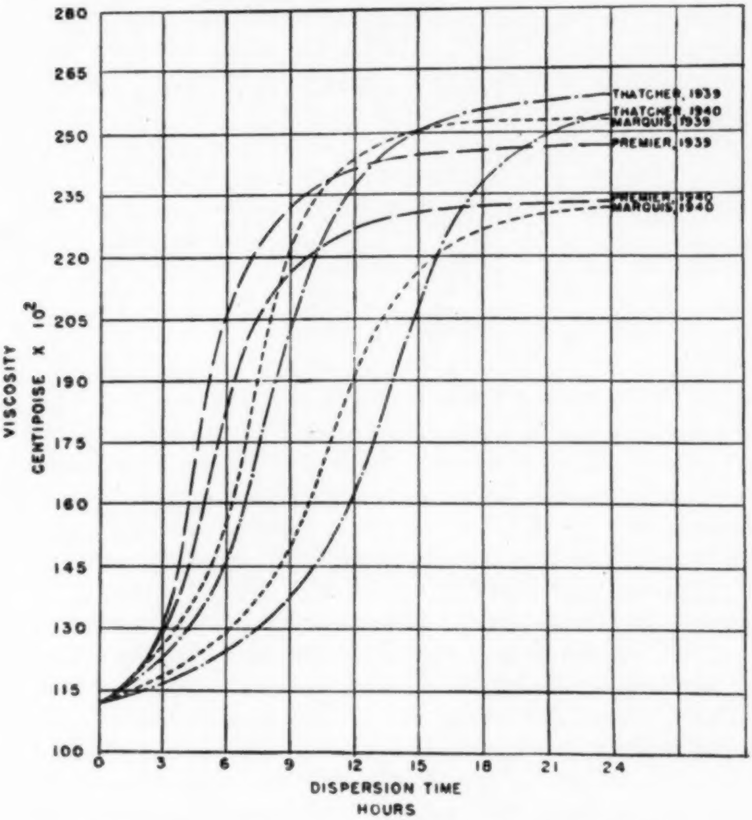

Fig. 9. Differences in gluten dispersion rate due to seasonal variability.

varieties tend to retain their characteristic placings when viscosity is plotted against time.

Table II presents correlation coefficients calculated between the more pertinent variables. Wheat protein and loaf volume are significantly related, but the correlation is too small to be of practical significance for prediction purposes. No doubt the large number of varieties contributed to the low magnitude of this statistic. Wheat protein and rate of dispersion are negatively correlated as would be expected from the observation that very strong wheats, which are usually high in protein, have glutens which tend, on the average, to

be more resistant to dispersion in sodium salicylate, while wheats of lower relative baking strength possess glutens which tend to disperse more rapidly than the average. These latter wheats exhibit trends toward lower protein content.

Specific volume of the protein micelle and loaf volume are not significantly correlated. This result is in agreement with conclusions

TABLE II

Correlation Coefficients Computed from the Data
(Significant correlation coefficients are in bold type.)

Variables correlated		Correlation coefficients	Probability
x	y	r_{xy}	P
Wheat protein, %	Loaf volume, *cc*	**+.3537**	.0038
Wheat protein, %	Rate of dispersion, *6 hrs*	**−.4002**	.0011
Specific volume, ϕ/C	Loaf volume, *cc*	+.2272	.0635
Rate of dispersion, *6 hrs*	Loaf volume, *cc*	−.0482	>.5485
Viscosity at 6 hrs	Viscosity at 24 hrs	−.2296	.0607
Viscosity at 6 hrs	Viscosity at 8 hrs	**+.9028**	<.0001

reached by Harris and Johnson (footnote 2) who postulated no correlation between baking quality and specific volume in varieties belonging to the hard wheat classes. Rate of dispersion is apparently not related to loaf volume, as obtained by the baking method used in this investigation. As some of the wheat glutens examined, which dispersed rapidly, had a relatively low viscosity after 24 hours of dispersion, it was thought desirable to determine whether there was a general relationship between these gluten characteristics and, accordingly, the correlation coefficient was computed between viscosities at the 6th and 24th hours. No significant interdependence was found, however.

The relationship between viscosity at 6 and 8 hours is very high and for practical purposes would justify the prediction of one variable from the knowledge of the other. As better differentiation between varieties is shown by the 6-hour results, it would seem that the latter value is of more general utility than the value obtained at any other time.

Because wheat protein and loaf volume, as well as wheat protein and rate of dispersion, are significantly correlated, the partial correlation coefficient between rate of dispersion and loaf volume was calculated, wheat protein being held constant. A value of +.1081 was obtained, which is below the level of significance.

Although no significant correlation was found between viscosity data and loaf volume, it is felt that a substantial amount of information respecting the gluten properties of the hard red spring wheats studied was gained. Thatcher is universally accepted as a variety

extremely high in strength, with a somewhat harsh, tough gluten, which requires a long mixing time to condition properly. This wheat invariably has a low gluten dispersion rate, usually the lowest of any variety. Premier and Mercury, on the other hand, lack the residual strength found in Thatcher, and their glutens are much less harsh and elastic. These varieties produce glutens which disperse rapidly in sodium salicylate. Other varieties which are intermediate in strength have glutens which disperse more easily than Thatcher but are more resistant than Mercury or Premier. Rival is one of these wheats. It appears probable that hard red spring wheats which possess glutens that disperse comparatively rapidly in sodium salicylate should be viewed with suspicion by the cereal technologist, at least until an extensive study has been made of their baking behavior under varying conditions of formula and method.

TABLE III

ANALYSIS OF VARIANCE OF DISPERSION RATES (DV/DT) OF ELEVEN WHEAT VARIETIES GROWN AT FOUR STATIONS IN NORTH DAKOTA IN 1940

Source of variation	Sums of squares	Degrees of freedom	Variance	F	5% point	1% point
Between stations	357.554	3	119.185	42.61	2.92	4.54
Between varieties	214.156	10	21.416	7.66	2.16	2.98
Interaction (stations × varieties)	83.906	30	2.797			
Total	655.616	43				

Table III shows the analysis of variance of the rates of dispersion at six hours for the eleven wheat varieties grown at four stations in 1940. In this table the variance calculated for interaction has been used as a criterion for estimating the significance of the variances existing between stations and between varieties. It measures the variation not attributable to either station or variety, its source being in the differential response of the station dispersion rates to wheat varietal trends. If the station averages had varied directly for each variety by precisely the same amount, the resultant interaction would have been zero. Another means of measuring the significance of the two variances would have been to use the differences and sums of duplicate determinations of viscosity, but since these readings were very accurate and were repeated if they differed by more than a set limit, it was thought that the interaction variance would be best to use rather than the variance calculated for the experimental error.

The variance for between stations is very large and gives a high F value. The between-varieties variance is smaller, but still extremely significant at both the 5% and 1% points.

The evidence presented in this table fully substantiates the conclusions derived from an inspection of the data given in the tables and figures already discussed.

It is evident that both wheat variety and environmental factors during growth, probably while the gluten proteins are being laid down, have a marked influence upon the gluten complex. This effect is manifested in the comparative rates at which the gluten disperses in 10% sodium salicylate, and is apparently related to certain "quality" characteristics of the gluten. It appears logical to assume that the structure of the complex has been altered in some manner by these influences and this change is reflected in the ease with which the gluten particles are dispersed. It is probable that changes take place in the manner in which the constituent portions of the complex are united. Wheats whose glutens disperse easily would have the constituent proteins less rigidly held together than in the instance of wheats possessing glutens which are resistant to dispersion. These differences in constitution would be shown in certain properties which are concerned with the "feel" of the gluten during washing, and in reactions of the gluten during the baking process. Factors which favor high wheat protein content have a tendency to increase gluten resistance to dispersion, probably by increasing the tenacity of bonds within the gluten complex. These changes do not affect the size, or hydration, of the gluten micelle to any marked degree in hard red spring wheats.

Summary and Conclusions

An investigation conducted upon 68 samples of hard red spring wheat, comprising 13 varieties grown at 6 North Dakota stations in 1939 and 1940, appears to justify the following conclusions:

The rate at which gluten washed from hard red spring wheat flour disperses in 10% sodium salicylate solution is related to wheat variety.

This dispersion rate is also related to the environmental conditions of soil and climate under which the wheat is grown, and accordingly the different stations produce wheats having significantly different rates.

A corollary to the statement respecting differences induced by environmental variations is that wheats grown in different years have glutens which disperse at different rates.

The differential quotient dv/dt appears to have the more significant value at the 6th hour of dispersion, owing to changes in the rates occurring at later periods of the dispersion process. In some instances the rate begins to fall off.

The specific volume ϕ/C of the gluten micelles in sodium salicylate dispersion is not significantly correlated with wheat protein content or loaf volume in hard red spring wheat varieties. This conclusion is in agreement with the hypothesis of Harris and Johnson (footnote 2), who found a significant relationship between specific volume and loaf volume when a number of wheats belonging to different classes were examined, but postulated no correlation within classes.

Glutens prepared from hard red spring wheats of high protein content tend to disperse more slowly than wheats of lower protein content.

Despite lack of relationship between the viscosity data and baking strength as registered by loaf volume, it is felt that valuable information respecting varietal and environmental gluten differences was obtained by determining the changes in viscosity of wheat gluten dispersion in 10% sodium salicylate between 0 and 6 hours of dispersion time.

Acknowledgments

The authors wish to acknowledge the valuable contributions made by L. D. Sibbitt in conducting the milling and baking determinations required in this investigation. Credit is also due to T. E. Stoa, Station Agronomist, who furnished the wheat samples, as well as to the NYA, and WPA Seed Testing Project, O.P. 165-1-73-144, for technical assistance.

THE COMPARATIVE BAKING QUALITIES OF HARD RED SPRING WHEAT STARCHES AND GLUTENS AS PREPARED BY THE GLUTEN-STARCH BLEND BAKING METHOD[1]

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(Received for publication September 29, 1941)

A number of papers have been published recently dealing with various methods of adding wheat gluten and starch to flour, or of mixing these constituents with each other, to form blends at a uniform protein level. These blends were then baked and the loaves examined in respect to comparative baking quality. By this method differences in baking strength caused by variations in protein quantity would be ruled out.

Aitken and Geddes (1938) raised the gluten content of a series of wheat flours by the addition of dried ground gluten and baked the doughs made therefrom. The resultant loaves closely resembled those

[1] Published with the approval of the Director of the Station.

produced by flours of corresponding natural protein content. The glutens were prepared from different wheat varieties and classes. With the exception of some very weak wheats (such as German and English) the gluten quality appeared to be fairly constant.

The senior author, in collaboration with H. N. Bergsteinsson,[2] built up the protein content of a soft-wheat flour dough by incorporating freshly washed wheat gluten in the dough mix. Marked improvement in loaf volume, external appearance, and internal characteristics were caused by the addition of the gluten.

Later Harris (1940) employed a method essentially resembling the procedure of Aitken and Geddes, wherein dried ground gluten was added to a series of flours milled from hard red spring, hard red winter, soft red winter, and durum wheats. The blends were then baked at a constant protein level. Hard-wheat glutens improved the loaf volume of the soft-wheat base flour used, while durum gluten tended to decrease the loaf volume. The average color and texture scores were raised by the addition of the dried gluten. The malt-phosphate-bromate baking formula was used in the three investigations mentioned above.

Sandstedt, Jolitz, and Blish (1939) showed the feasibility of baking bread from blends of dried gluten and starch prepared from wheat flour. Satisfactory loaves were produced, and the authors stated that the blend loaves closely resembled those baked from the original flour. Harris and Sibbitt (1941) applied this method to the determination of the baking quality of a series of starches prepared from different classes and varieties of wheat. A common gluten substrate washed from hard red spring wheat was used and suitable proportions of starch were then superimposed upon it. Three protein levels (10.0%, 13.2%, and 16.0%) were employed. The results of this investigation showed that starches prepared from different wheats produced loaves differing greatly in volume, general appearance, and internal characteristics.

Sandstedt and Ofelt (1940) diluted a series of hard red winter wheat flours with starch prepared by the method used by Sandstedt. Jolitz, and Blish (1939). A constant protein level of 10.0% was used, and the diluted flours were baked by a series of formulas. The data obtained indicated great variability in protein quality among varieties. Within varieties protein quality also varied with the original wheat protein content. Fortmann and Sandstedt[3] extended this series of protein quality investigations. Ten wheat varieties grown at 14 Nebraska locations were examined. It was found that any one variety

[2] At present, research chemist, Lake of the Woods Milling Company, Keewatin, Ontario.
[3] Karl Fortmann and R. M. Sandstedt: Effect of environment and variety on wheat flour quality. Read at A. A. C. C. meeting in Omaha, 1941.

grown in different localities showed striking differences in flour quality. It was also shown that varieties grown in any particular environment had much the same relative baking qualities in reference to each other as the same varieties had when grown under other environments. It is essential when evaluating varieties that they be grown under identical conditions.

In view of the results obtained in the investigations cited, it was thought desirable to study the effects of wheat variety and location of growth upon the baking results obtained from a series of starch-gluten blends made from hard red spring wheat starch and gluten. In this way the influences of variety and locality upon wheat starch and gluten could be examined separately by blending starches prepared from various varieties of commercial importance with a constant gluten substrate, and then reversing this plan and baking different glutens with a constant starch.

Experimental Material and Methods

Forty-eight samples of starch were prepared from 13 varieties of hard red spring wheat by a method which was essentially that used by Sandstedt, Jolitz, and Blish (1939). Eleven of these varieties were grown in replicated plots under comparable conditions at four different stations in North Dakota in 1940. The remaining two varieties were grown at only two stations. They were free from any damage that might tend to influence the results with respect to varietal or environmental differences. The wheats were milled into straight-grade flour on an Allis-Chalmers mill under laboratory conditions of approximately 70°F temperature and 60% relative humidity. The glutens washed from these flours were divided into pieces averaging ½ g in weight, placed on waxed paper sheets, and dried at 90°F for 24 hours. During the first 8 hours the relative humidity of the drying cabinet was gradually reduced from 94% to room conditions (approximately 50%). The sheets were hung parallel to the air current in the drier. The dry gluten pellets were removed from the sheets and reduced to a powder in a suitable grinder.

The starches and glutens were analyzed for moisture and total protein content. From the data the respective proportions of the two ingredients required to furnish a protein content of 13.2% on a 13.5% moisture basis were calculated. The procedure employed in mixing the blends was to hydrate the gluten for one hour by the addition of a constant volume (12 ml) of distilled water at 30°C, and then add the hydrated gluten plus any unabsorbed water to the dry starch in the mixer bowl. The Hobart mixer with two dough hooks was used to mix the doughs. A total mixing time of 2½ minutes minus 15 seconds

for scraping down the pins and sides of the bowl was used. The formula was the malt-phosphate-bromate with 7% sucrose. The micro baking procedure was used with standard fermentation periods, as described by a number of investigators (Geddes and Aitken, 1935; Harris and Sanderson, 1938; VanScoyk, 1937, 1939). The loaf volumes were determined 30 minutes after baking by a calibrated micro loaf volumeter. The original flours were baked by the 100-g method using the Hobart-Swanson and the malt-phosphate-bromate formula. The morning after baking, all loaves were examined under a fluorescent light for crust color, external appearance, and internal characteristics.

Discussion

The varieties included in the study were those of particular interest to the northwestern hard red spring wheat area, comprising several of the older wheats which have been grown in this region until they have come to be considered more or less as standards with which newer varieties may be compared in respect to agronomic and quality factors. Included also were some wheats which have been recently released for growth within the state, as well as a number that are being tested with a view to later distribution. Members of the first group, the older tried wheats, are Marquis, Ceres, and Thatcher, while varieties that have been recently introduced into the variety picture are Pilot B, Rival, and Nordhougen.[4] Wheats under trial are Merit, Pilot 13, Vesta, and Premier. The remaining varieties are those which have originated in Canada and may be grown to some extent in the northwestern states if they prove satisfactory.

The four stations at which the wheats were grown are located in the eastern, central, western, and northeastern sections of the state and fairly well cover the principal portion of the wheat-producing area of North Dakota.

Some differences in test weight per bushel were evident in the data, but these are not more than would be expected where a number of wheat varieties are grown under variable environmental conditions. The wheat protein ranged from 13.0% to 16.6%, and the flour yield from 60.2% to 75.7%. The ash values were normal for experimental flours milled from hard red spring wheat varieties. Some variability was evident in the starch and gluten moistures but these differences were not related to wheat variety. It was found that moisture content varied more among starches than among glutens, a result in all probability of more constant conditions when the latter material was dried. The starch moisture ranged from 6.7% to 12.1%, while the gluten varied from 8.3% to 10.6%. The protein content of the

[4] This wheat was developed by a private grower and is being produced to some extent in the state.

starches was quite uniform, differing by not more than 0.2%, but the protein content of the dried glutens varied from 63.5% to 75.5%. The glutens washed from wheats grown at the Dickinson Station were highest in protein content.

The comparative baking data obtained on the original flours and the starch-gluten and gluten-starch blends led to the following conclusions. The absorption of the blends was higher owing principally to their lower moisture content as compared with the original flours. The loaf volumes obtained on the flours cannot be directly compared with the corresponding values of the blends owing to the facts that the protein contents were different in the original flours and a different baking method was used for the flours than was employed for the blends. The flour absorptions and loaf volumes showed marked variability. The absorption range was from 55.3% to 60.7% and the loaf volume range from 525 cc to 720 cc. There were also substantial differences in crumb color scores, which varied from 6.2 to 8.5.

TABLE I

STATISTICAL CONSTANTS COMPUTED FROM THE DATA

Variable	Mean	Minimum	Maximum	Range	Standard deviation	Coefficient of variability
						%
Absorption, original flours, %	58.2	55.3	60.7	5.4	1.507	2.59
Absorption, starch-gluten blends, %	68.3	66.6	70.6	4.0	1.119	1.64
Absorption, gluten-starch blends, %	64.3	61.6	67.6	6.0	1.355	2.11
Loaf volume, starch-gluten blends, *cc*	131.0	114.0	145.0	31.0	8.633	6.59
Loaf volume, gluten-starch blends, *cc*	131.3	106.0	160.0	54.0	11.939	9.09
Crumb color, original flours	7.4	6.2	8.5	2.3	0.598	8.08
Crumb color, starch-gluten blends	7.0	6.5	7.5	1.0	0.343	4.90
Crumb color, gluten-starch blends	7.1	5.5	8.0	2.5	0.769	10.83

The absorptions in the starch-gluten blends were less variable than in the case of the original flours and ranged from 66.6% to 70.6%, a difference of 4% as compared with 5.4% for the flours. The loaf volumes ranged from 114 cc to 145 cc. The crumb colors varied from 6.5 to 7.5, a very small difference. It was evident that loaves baked from the starch-gluten blends, prepared in the manner described, do not show the differences in characteristics to be found among the loaves baked from the original flours themselves. This result is to be expected, since some of the sources of variability have been removed. The protein content is uniform, while a constant gluten substrate has been employed throughout the series, thus obviating differences due to possible variations in gluten quality. Other causes of difference may be found in the removal of certain flour constituents, as enzymes,

lipids, inorganic salts, etc., during the washing of the starch and gluten. Despite the removal of certain proportions of these constituents, however, there can be little doubt that differences in results have been caused by inherent variability in the starches themselves. This variability may have been due to factors such as differences in starch damage during milling, or to other causes.

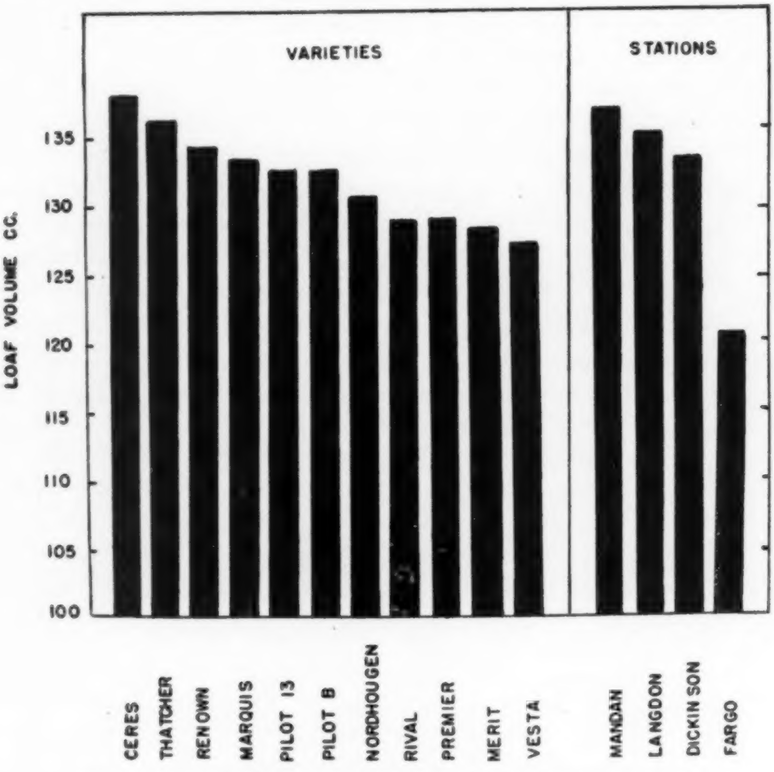

Fig. 1. Comparative loaf volumes obtained by baking blends of starch with a common hard red spring gluten substrate.

The absorption values of the blends made with a variable gluten superimposed upon a constant starch base were lower than the starch-gluten values, but higher than those of the flours themselves. They varied from 61.6% to 67.6%, a difference of 6.0%, as compared to 5.4% for the flours and 4.0% for the starch-gluten doughs. The loaf volumes ranged from 106 to 160 cc, a difference of 54 cc, while there was a difference of only 31 cc in the starch-gluten blends. The crumb colors varied from 5.5 to 8.0, or a difference of 2.5 as contrasted with 1.0 for the starch-gluten loaves and 2.3 for the original flours. These comparative results are presented in condensed form in Table I, which

gives means, minima and maxima, ranges, standard deviations, and coefficients of variability for the different sets of baking data.

It is apparent that the mean loaf-volume values for both series of blends were the same. There was also more variability in loaf volume in the gluten-starch than in the starch-gluten blends. The crumb color

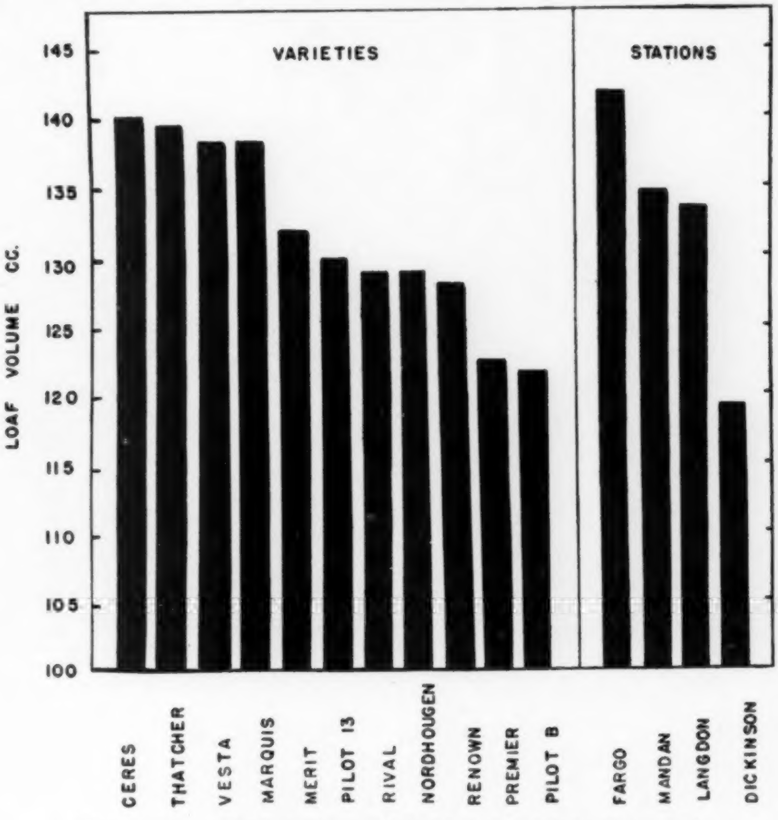

Fig. 2. Comparative loaf volumes obtained by baking blends of gluten with a common hard red spring starch substrate.

in the gluten-starch blends was more variable than in the other two series of bakings, while it was least variable in the starch-gluten blends.

For the purpose of making comparisons of starch and gluten baking qualities among wheat varieties and stations, the loaf volume data from the eleven varieties which were grown at all the stations were next examined. The comparative loaf volumes obtained on the starch-gluten blends are shown in Figure 1. The data are arranged in order of decreasing loaf volume from left to right. Ceres wheat starch produced the largest loaf in this series, while Thatcher starch gave the next largest. Rival, Premier, Merit, and Vesta starches gave the

lowest loaf volumes. Both varieties of Pilot were equal in starch baking quality and are in the same range as Marquis starch. Variation in mean loaf volumes among stations was more pronounced than among varieties, Fargo being substantially below the other three stations, which gave very similar results.

Figure 2 shows similar data for the gluten-starch blends. Larger differences among varieties are apparent than were found in the case of the starch-gluten blends. Ceres, Thatcher, Vesta, and Marquis are similar in results obtained by baking their glutens with starch as

TABLE II

Analysis of Loaf-Volume Data

Source of variance	Sum of squares	Degrees of freedom	Variance	F	5% point	1% point
ANALYSIS OF VARIANCE OF STARCH-GLUTEN LOAF VOLUMES						
Between stations	1814.97	3	604.99	25.84	2.92	4.51
Between varieties	551.00	10	55.10	2.35	2.16	2.98
Interaction (stations × varieties)	702.28	30	23.41			
Total	3068.25	43				
ANALYSIS OF VARIANCE OF GLUTEN-STARCH LOAF VOLUMES						
Between stations	2910.91	3	970.30	14.92	2.92	4.51
Between varieties	1611.23	10	161.12	2.48	2.16	2.98
Interaction (stations × varieties)	1950.59	30	65.02			
Total	6472.73	43				

described. An intermediate group consists of Merit, Pilot 13, Rival, Nordhougen, and Renown, while Premier and Pilot B fall into the lowest group. There is a substantial difference between the mean blend loaf volumes for Dickinson and the other three stations. In this series of bakings the Fargo wheats yielded the largest loaves, while Mandan and Langdon were the same.

The data from the two series of blends were analyzed to determine the significance of the differences of the mean blend loaf volumes among the various varieties and stations. The results obtained from the two series of blends are shown in Table II. The variance for interaction (stations × varieties) was used to measure the significance of the variances due to station and variety differences. The interaction variance arises from the differential response of the station loaf volumes to the varietal trends.

The significance of the differences between station and variety

means may be determined by the use of the standard error s.

$$s = \sqrt{23.409} = 4.838 \text{ in the case of the starch-gluten blends.}$$

Then $s_m = 4.838/\sqrt{4} = 2.419$, and the standard error of the difference between the means of any two varieties is then $2.419 \times \sqrt{2} = 3.42$. For Thatcher and Vesta, the difference between means is 9.0 cc. Therefore, $t = 9.0/3.42 = 2.63$. This is a significant difference inasmuch as t at the 5% point is 2.04.

To determine the significance of the difference between station means the same technique is used, except that s_m is now $4.838/\sqrt{11} = 1.458$, as there were 11 varieties grown at each station.

The significance of the differences in mean gluten-starch loaf volumes may be found in a similar manner.

From the results enumerated it would appear that the chief source of variability in baking quality when the protein content is held constant lies in the properties or "quality" of the gluten proteins. This conclusion is in agreement with the general consensus of opinion among wheat technologists and corresponds with the postulate of Sandstedt and Ofelt (1940) and Fortmann and Sandstedt (footnote 3). Harris and Sibbitt (1941) have indicated that very substantial differences in baking results may be brought about by varietal effects upon starches used in a baking blend with constant gluten menstruums. Their work was done with wheats belonging to different classes, however, and this fact would partly account, no doubt, for the larger differences in starch baking quality which they found.

Summary and Conclusions

Forty-eight samples of starch and gluten were prepared essentially by the procedure reported by Sandstedt, Jolitz, and Blish (1939) from 13 varieties of hard red spring wheat grown at four stations in North Dakota. The starches were blended individually with a common gluten substrate prepared from hard red spring wheat, and made into doughs and baked. Similarly, the different glutens were blended with a hard red spring wheat starch and baked. These two series of bakings were done at a constant protein level. The standard basic formula with 7% sucrose was employed, with the micro baking method. The results obtained appeared to justify the following conclusions:

Doughs made from starch-gluten blends are higher in absorption than the original flour doughs. This is caused largely by the lower moisture level of these four constituents after drying. The absorption of doughs made from a variable starch with constant gluten was higher than that of doughs made with a variable gluten and constant starch. The reason for this difference is not clear.

The crumb-color score of the bread made from the original flours was higher than the blend-loaf score.

Significant differences in loaf volume of the blends were shown between varieties of wheat, as well as between locations of growth, by the analysis of variance. It would follow that both the starches and glutens prepared from hard red spring wheats by the method used in this study vary in baking quality as a result of both inherited and environmental factors. These differences, as far as variety is concerned, tend to show that certain wheats which have been viewed with suspicion in respect to baking strength have glutens which yield loaves of inferior volume as compared to glutens washed from wheats commonly considered to be of superior baking strength. Some of these superior wheats apparently have starches which produce loaves of relatively high quality when used in starch-gluten blends.

Because of the small number of samples of each variety included in the study it was not possible to draw conclusions regarding the effect of original wheat protein content upon gluten or starch baking strength within a variety.

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THE EFFECT OF TEMPERATURE UPON THE VIABILITY AND BAKING PROPERTIES OF DRY AND MOIST YEAST STORED FOR VARIED PERIODS

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It frequently becomes necessary to store yeast in small or large quantities for a time before use, particularly in isolated parts of the country. The best methods for the preservation of yeast, as with other perishable products, are of especial importance in the present war program when transportation facilities are being curtailed.

A review of the literature reveals that considerable experimental testing has been carried on with regard to the viability of different brands of moist yeast, both fresh and stored. No published material was found on the viability of dry yeasts, which are commonly used for home baking in rural areas.

Iwanowski and Brezezinski (1934) found that moist yeast could be stored at 32°F for two to three months without much loss in activity, whereas 10% to 20% of the cells were dead in yeast stored at 56°F for about two weeks and 20% when stored at 72°F for one week.

Bohn and Favor (1939) compared five brands of moist yeast by means of the pressuremeter and found variations in the amount of gas liberated from hour to hour by the different brands. Doty and Urban (1940) correlated the rate of gas production in moist yeast with the texture and crust characteristics of bread. The quantity of gas produced during the fourth hour gave a good picture of what the yeasts would do in a dough.

Weaver, Talbott, and Coleman (1933) reported that during aging there was a marked change in the general appearance of moist yeast. One brand retained its leavening qualities without impairment for 96 hours, with only slight changes at the end of 168 hours. Bread from the other brand showed a marked decrease in loaf volume, grain, texture, and crumb color scores after the yeast was stored 48 hours.

Bailey, Bartrom, and Rowe (1940) made bread with frozen moist yeast. They reported that the temperature of evaporated dry ice (−109°F) injures yeast in 24 hours and subsequent storage below freezing increases the amount of deterioration. The most satisfactory storage temperature was reported as 30°F, although comparatively good bread was made with frozen yeast stored a month or two at 20°F.

Experimental Procedure

In the experiments reported in this study, the viabilities of both dry and compressed yeast have been investigated. Dry yeasts secured from the factory were separated into lots and placed in air-tight glass containers. A part of each was stored in the refrigerator with temperatures ranging from 40° to 50°F and a part at room temperatures of 70° to 75°F. Compressed yeast was tested fresh and frozen. The frozen yeast was stored from one to twelve months. The dry yeast was stored for as long as 32 months.

The Sandstedt-Blish pressuremeter was utilized to measure periodically the gas produced by these yeasts in a dough. The formula with compressed and quick-action dry yeast consisted of 0.3 g yeast (3% based on flour), 0.574 g sucrose, 10 ml water, and 10 g flour. The formula with dry yeast cakes, which required a considerably longer fermentation for an equal degree of lightness, consisted of 0.6 g yeast, 0.574 g sucrose, 2 g mashed potatoes, 8 g flour, and 10 ml water. Several formulas were tested with the fresh dry cakes in order that the fermentation could be speeded up sufficiently to secure, over an eight-hour period, pressuremeter readings which approximated those secured with moist yeast in a dough fermented four hours. Doubling the amount of yeast and the addition of mashed potatoes aided in this. Potatoes are quite generally used in home baking with dry yeast cakes.

The quantity of sugar necessary was calculated according to the method of Sandstedt (private correspondence) by the formula 1000—(4/3 gassing power) = milligrams of sugar to be added, 4/3 gassing power being the quantity of sugar developed by diastasis in the flour in the course of a four-hour fermentation. In the recorded tests with the various yeasts, one flour was used for the doughs.

The fermentation was carried on at a constant temperature of 86°F. Readings were recorded and the gas in the pressuremeter was released at the end of each hour. The gas was allowed to escape slowly, so that the cooling effect due to adiabatic expansion would not affect the readings.

The viability of the moist yeast was further tested by staining with methylene blue, which stains the dead and injured but not the living cells.

Results

Quick-action dry yeast: The pressuremeter readings during the fermentation of the quick-action dry granulated yeast stored under the conditions noted are given in Table I. It is evident from the table that the loss of activity was considerably greater for this yeast

TABLE I

PRESSUREMETER READINGS DURING FERMENTATION OF QUICK-ACTION DRY YEAST STORED FOR VARIED PERIODS OF TIME AND AT DIFFERENT TEMPERATURES

Quick-action dry yeast stored at 40°–50°F			Quick-action dry yeast stored at 70°–80°F		
Time in storage	Gas	Loss in activity	Time in storage	Gas	Loss in activity
	mm Hg	%		*mm Hg*	%
Fresh	810	—	Fresh	810	—
1 month	770	4.9	1 month	720	9.9
6 months	625	22.8	7 months	510	37.0
12 months	520	35.8	12 months	390	51.9
31 months	425	47.5	18 months	230	71.6

stored at room temperature than for the same lot stored in the refrigerator, and also that its activity became consistently less as the time in storage was prolonged. Differences in the quality of the bread made with quick-action dry yeast stored at different temperatures for varied times are shown in Figure 1.

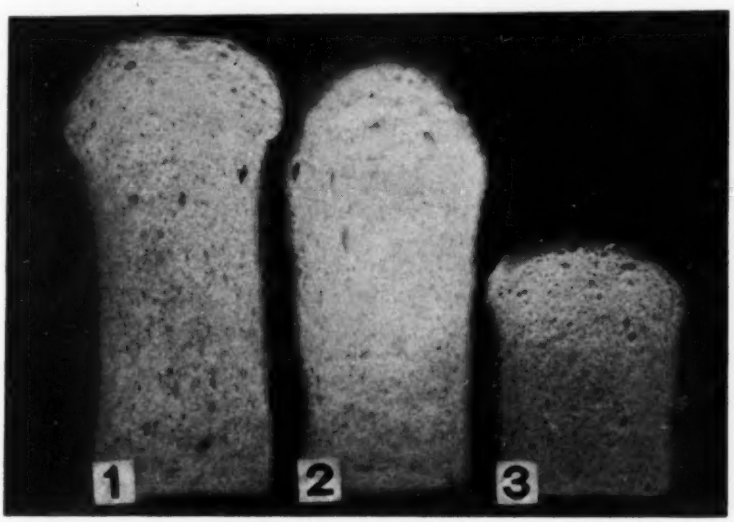

Fig. 1. Loaves made from quick-action dry yeast. Loaf No. 1 was made with the fresh yeast, loaf No. 2 with yeast stored in the refrigerator at 40°–50°F for 12 months, and loaf No. 3 from the same lot of yeast stored at a room temperature of 70°–80°F.

In Figure 1, loaves 2 and 3 were made from the same lot of yeast, but for loaf No. 2 the yeast was stored at 40° to 50°F, while for No. 3 it had been stored at room temperature for twelve months. The extremely poor quality of loaf No. 3 as compared with No. 2 makes it evident that the length of time this yeast can be stored satisfactorily depends in a large measure upon the temperature of storage.

Each loaf shown in Figure 1 contained 2 g of quick-action dry yeast and 340 g of flour, together with sugar, salt, fat, and water in weighed amounts. The total fermentation of the dough of loaf No. 3 was 50 minutes longer in an attempt to lighten it sufficiently for baking.

Dry yeast cakes: The activity of dry yeast cakes, used with the formula specified and fermented in the pressuremeter for eight hours, is recorded in Table II. This type of yeast is rapidly being replaced

TABLE II

PRESSUREMETER READINGS DURING THE FERMENTATION OF DRY YEAST CAKES STORED FOR VARIED PERIODS OF TIME AND AT DIFFERENT TEMPERATURES

Dry yeast cakes stored at 40°–50°F			Dry yeast cakes stored at 70°–85°F		
Time in storage	Gas	Loss in activity	Time in storage	Gas	Loss in activity
	mm Hg	%		*mm Hg*	%
3 days	552	—	3 days	552	—
4 months	540	2.2	4½ months	230	58.3
10 months	338	20.6	6 months	185	66.5
15 months	260	52.9	8 months	90	83.7
32 months	185	66.5	17½ months	25	95.4

by those of speedier action. It is, however, still used to a considerable extent for home baking in rural districts. The superiority of cold temperatures for the preservation of dry yeast cakes is strikingly evident, as can be noted from the data and from the bread baked from this yeast as shown in Figure 2.

In Figure 2, loaves No. 1 and No. 2 became light with almost equal speed in the dough but No. 3 was fermented 2½ hours longer in an attempt to increase its lightness sufficiently for baking. With other lots the loss varied somewhat from the above figures but followed the same trend. Each loaf contained 4 g of the dry yeast cake, 15 g of mashed potato, and 100 g of flour fermented in a sponge over night in a proofing cabinet at 70°F. An additional 240 g of flour together with fat, sugar, salt, and water in weighed amounts was added the following morning. The dough was fermented and pan proofed as with the quick-action yeasts. The length of time the dry yeast cakes can be stored and retain sufficient viability for good bread depends upon the temperature of storage.

Moist yeast: The viability of three lots of moist yeast in a fresh and frozen condition was measured.

It has long been recognized that cold temperatures are essential to keep moist yeast in good condition. Bailey, Bartram, and Rowe (1940) reported that temperatures slightly above the freezing point

or about 30°F were satisfactory for continued storage. It is conceivable that this temperature might be difficult to maintain when yeast is transported or stored at home. The temperatures in household mechanical refrigerators are usually maintained around 40°–50°F for ordinary refrigeration. At such temperatures moist yeast soon becomes discolored and can be preserved for only short periods.

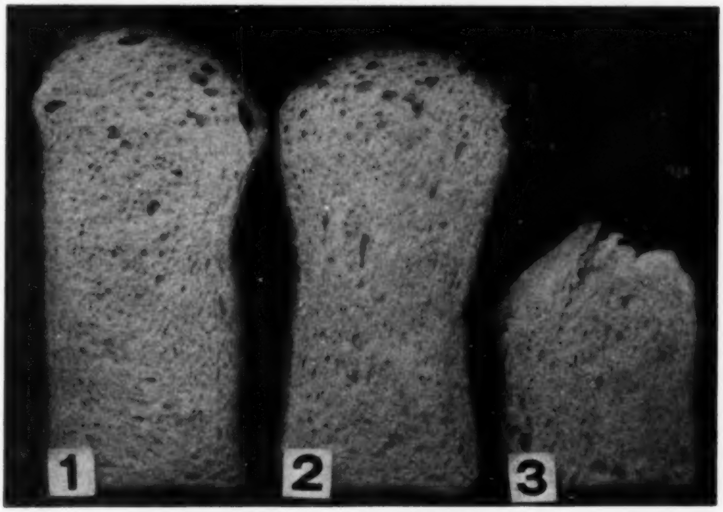

Fig. 2. Loaves made from slow-action dry yeast cakes. Loaf No. 1 was made from the fresh dry cake, loaf No. 2 from a cake stored in the refrigerator at 40°–50°F for 12 months, and loaf No. 3 from the same lot of yeast stored at a room temperature of 70°–80°F.

Freezing was considered the most convenient method for the storage of moist yeast in isolated districts and was therefore used in these tests. Frozen moist yeast may be conveniently stored in freezer lockers in rural districts. The freezing units in household refrigerators may be utilized for short storage periods. Precautions must be taken that the yeast is not allowed to thaw during the storage time. The activity of the frozen yeast stored for varied periods at 19°F, the temperature at which the home refrigerator freezing unit is often kept, was measured in the pressuremeter and is recorded in Table III.

It is evident from Table III that the compressed yeast frozen for a month liberated almost a normal amount of gas with surprisingly slight losses after being frozen three or four months. Its activity was measured by testing portions of the same cake from each of the above lots after these varied storage periods. Yeast from these lots was also tested in bread from time to time.

All test loaves made with moist yeast contained 7 g of yeast and 340 g of flour, together with fat, sugar, salt, and water in weighed

TABLE III

PRESSUREMETER READINGS ON THREE LOTS OF FROZEN COMPRESSED YEAST STORED FOR VARIED PERIODS

Lot I			Lot II			Lot III		
Time kept frozen	Gas	Loss in activity	Time kept frozen	Gas	Loss in activity	Time kept frozen	Gas	Loss in activity
	mm Hg	%		*mm Hg*	%		*mm Hg*	%
Fresh	500	—	Fresh	505	—	Fresh	500	—
4 wks	495	1.0	8 wks	500	1.0	4 wks	497	0.6
8 wks	490	2.0	12 wks	490	3.0	10 wks	493	1.4
12 wks	490	2.0	20 wks	475	5.9	13 wks	493	1.4
16 wks	475	5.0	26 wks	385	23.8	18 wks	492	1.6
24 wks	410	18.0				24 wks	420	16.0
40 wks	340	32.0	44 wks	320	36.6	48 wks	180	64.0
48 wks	320	36.0						

amounts. The fermentation was carried on in a proofing cabinet with temperatures constant. The total time required for fermentation and pan proofing of the dough made from one of the lots of yeast frozen for a month was approximately 20 minutes more than was necessary to reach the same degree of lightness with fresh yeast.

Fig. 3. Loaves made from compressed yeast. Loaf No. 1 was made from fresh yeast, loaf No. 2 from yeast frozen one month, and loaf No. 3 from yeast frozen 3 months.

Bread from another lot of yeast frozen three months fermented about 60 minutes longer, while that from two lots frozen 11 to 12 months required around 2½ hours of additional time. Yeast from a third lot frozen 12 months did not lighten the dough sufficiently for baking. The doughs were fermented in calibrated expansion tubes and pan

proofed in uniform test pans. Loaves from fresh compressed yeast and from yeast frozen for one and three months are shown in Figure 3.

It is evident from Figure 3 that the quality of bread made from the frozen yeast was good, as is also indicated by the judges' scores in Table IV. A lowering of quality was noted in the bread made from yeast frozen for long periods. Although these loaves required longer fermentation to reach the same degree of lightness, they were still fair in quality, as can be noted from their ratings and from the loaves shown in Figure 4.

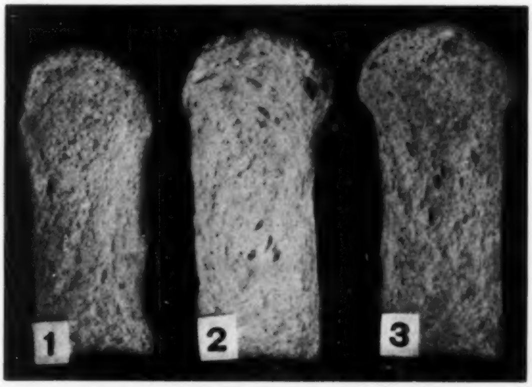

Fig. 4. Loaves made from frozen compressed yeast. Loaf No. 1 was made with yeast frozen 4 months, loaf No. 2 with yeast frozen 11 months, and loaf No. 3 from yeast frozen 12 months.

Bailey (1940) reported somewhat greater deterioration in frozen yeast stored at 20°F than was evident in the Wyoming tests, which may have been due to differences in the viability of the yeast or to methods of storage and handling. In Bailey's tests, pound cakes were frozen but were not in special wrappings or containers. A part of the yeast was thawed over night before being mixed into a dough. In the tests reported here, small cakes were left wrapped in foil, and after opening, were rewrapped in waxed paper for continued storage. A part was stored in a practically air-tight and moisture-proof metal container in the freezing unit, and the remainder was left in the freezing tray. The frozen yeast was used immediately when removed. It was thawed in lukewarm water (83°F) before being mixed into a dough.

The extent to which the yeast cells survived the low temperatures when stored for varied time periods was further measured by staining with methylene blue, which stains the dead but not the living cells. The following technique was used: Suspensions were made from the fresh and also from varied lots of the stored and frozen compressed yeast by adding a weighed amount to a phosphate buffer according to

the recommendations of Fink and Kuhle (1933) and Mills (1941).[1] A buffer with a pH of 4.6 seemed most favorable for preventing an increase in the number of stained cells during the counting. Such an increase was evident with a pH value of 7 and above.

Methylene blue was added in an amount to make the dye concentration 1 : 10,000 as recommended by Mills (1941).[1] The staining was preferred to plate counts as it was much speedier. Moreover, plate counts, in Mills' opinion, gave the number of reproducing cells rather than a representative count of the viable cells in the fermenting culture.

One drop of each suspension was examined under the microscope and counts were made on a Thoma-Zeiss haemacytometer. The counts on each lot were made on about 100 small square areas or until the total averaged around 1200 cells. The formula for calculating the percent of dead cells by staining was

$$\frac{S}{U + S} \times 100$$

where S represents the number of stained cells and U the number of unstained cells.

The time interval had little effect on numbers of stained cells in the buffered solution, whereas in distilled water there was an increase of 28% after several hours.

TABLE IV

THE RELATIONSHIP OF THE NUMBER OF DEAD CELLS IN FROZEN YEAST TO PRESSUREMETER READINGS, FERMENTATION AND SCORING OF LOAVES

Sample	Condition	Age yeast	Dead cells	Pressure meter reading	Time required for dough fermentation and proofing	Baking value, basis of 100
			%	*mm Hg*		
1	Not frozen	Fresh	1.7	505	4 hr 30 min	95
2	Frozen	1 mo	6.0	495	4 hr 50 min	90
3	Frozen	3 mo	9.6	490	5 hr 33 min	90
4	Frozen	4 mo	11.03	450	5 hr 45 min	90
5	Frozen	11 mo	21.9	340	6 hr 50 min	85
6	Frozen	12 mo	25.0	320	7 hr	70

The loss of living cells, the lessening of the activity of the yeast in a dough, and the rating of the bread made from yeast frozen for varied times are given in Table IV.

A gradual loss in viability of the frozen yeast during storage is evident in Table IV. The data show that the yeast held up surprisingly well for several months. A loss of 10% to 14% of the cells

[1] Note Appendix.

seemingly did not slow up fermentation to the extent that the yeast was undesirable for bread. The longer proofing time up to a certain point, with a high-grade flour, did not seem to affect adversely either the flavor or quality of the loaf, as is evidenced by the scores in Table IV. The ratings were made by trained judges on unidentified loaves. The baking values indicate that bread of good quality was made from yeast frozen for periods up to four months.

Yeast from different lots showed considerable variability. Some lots withstood the long storage period at low temperatures better than others. Counts revealed that from 1.7% to 2.4% of the cells were stained in fresh cakes from several different lots purchased from the grocer and tested at once upon delivery, whereas 6.2% to 8.5% were stained in different lots frozen for two months. In one lot frozen a year 22% of the cells were dead, whereas in others frozen a similar time, 31% to 50% were stained.

The appearance and physical condition of yeast frozen over a period of time and stored in containers was much better than that kept in an open tray. A portion of the latter was dark, dry, and covered with mold. For the most part the lots of frozen yeast, if well wrapped, were in an excellent physical condition and retained their natural color. This yeast frequently became sticky and difficult to handle when thawed. One satisfactory method for handling was thawing in lukewarm water at 83°F. A second method, in which the yeast was allowed to thaw at room temperature over night before incorporation into a dough, proved less satisfactory. This was no doubt due to loss in viability after removal to the warm temperature, as shown in Table V.

TABLE V

Deterioration of Frozen Yeast after Thawing

Cake	Months frozen	Length of time left at room temperature upon removal from storage	Dead cells
			%
1	4 mo	Tested at once	11.03
1	4 mo	Four hours, unwrapped	12.8
1	4 mo	Twelve hours, unwrapped	33.8
1	4 mo	Eighteen hours, unwrapped	70.03

It is of interest to note that living organisms such as yeast can withstand freezing temperatures over a period of time and resume almost normal biological activity when placed under favorable environmental conditions, such as the incorporation of the yeast into a sponge or dough, with fermentation at 83° to 85°F. It is well known that high temperatures have the opposite effect.

It has been evident from this study that both dry and moist yeast may be stored at low temperatures over a period of months and still produce good bread. The viability of the stored yeast may be speedily measured from time to time by using the methods described.

Summary

Both dry and compressed yeast were active over much longer periods when stored at temperatures of 40° to 50°F than when stored at room temperatures of 70° to 75°F. The activity of the yeast was measured by fermenting in a Sandstedt-Blish pressuremeter and by bread baking tests. The pressuremeter method proved a speedy and excellent way to test the viability of both dry and moist yeasts.

Compressed yeast was well preserved for several months by freezing and resumed almost normal biological activity when mixed into a dough and placed under favorable environmental conditions.

Staining with methylene blue proved a speedy method for determining the viable cells in stored moist yeast. A phosphate buffer was added in order to prevent an increase in the number of stained cells during the counting.

Microscopic examination revealed a comparatively small percentage of dead cells in moist yeast frozen one to three months. The percentage increased with longer storage. A loss of 10% to 14% of the cells did not slow up dough fermentation to the extent that the yeast was unsuitable for bread.

Acknowledgments

The author is indebted to the Northwestern Yeast Company and Standard Brands, Inc., for yeast to carry on these tests and to Laurens Anderson, technical assistant, for preparation of the buffer and stain.

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APPENDIX

Buffer and Stain for Yeast Cell Counts

LAURENS ANDERSON, TECHNICAL ASSISTANT

A. The buffer was made $0.2M$ in NaH_2PO_4, the concentration suggested by Mills (1941). The concentration of Na_2HPO_4 required to give a pH of 4.6 was determined by experiment, and was found to be $0.004M$. At the same time the value of pK in the physico-chemical equation

$$\mathrm{pH} = pK + \log \frac{B}{A} \tag{1}$$

was computed, as the value of 6.8 given in the literature for the phosphate buffer system does not fit at these concentrations. In the above equation, B represents concentration of Na_2HPO_4 and A represents concentration of NaH_2PO_4, expressed in moles per liter.

Putting the experimentally determined values of pH and B into equation 1, with $A = 0.2$, and solving for pK we get 6.3 for this constant.

A suggested procedure for making this phosphate buffer is as follows (quantities for 650 ml buffer):

1. Make a saturated NaH_2PO_4 solution as follows: Dissolve 70 g $NaH_2PO_4 \cdot H_2O$ in 43 ml water by heating in a boiling-water bath. Filter the hot solution and place in constant-temperature bath at 25°C until crystallization begins, and let stand 12 hours after this. (Total time will be at least 24 hours.) Decant the solution from the crystals, and pipette 25 ml of the solution, free from crystals, into a 1,000-ml graduate.
2. Make a saturated Na_2HPO_4 solution in the same manner, dissolving 40 g $Na_2HPO_4 \cdot 12H_2O$ in 49 ml H_2O. Decant after crystallization, taking care that no crystals are in contact with the solution which is to be used.
3. Dilute the 25 ml of sat NaH_2PO_4 sol to 726 ml with water. Remove 80 ml of this solution.
4. Measure 35.36 ml of the saturated Na_2HPO_4 from a burette into a 50-ml volumetric flask, and dilute to the mark. This gives $0.6M$ Na_2HPO_4.
5. Add 4.33 ml of the $0.6M$ Na_2HPO_4 from a microburette to the 646 ml of dilute NaH_2PO_4 in the graduate.
6. Determine the pH of the resultant mixture. If the steps in the procedure so far have been carried out carefully, the buffer should have a pH of 4.6, and should have the composition: $NaH_2PO_4 = 0.2M$, and $Na_2HPO_4 = 0.004M$.
7. (*a*) If the pH is less than 4.6, add more Na_2HPO_4 solution, calculated as follows:

Let the amount to be added, measured in ml, $= C$, then

$$C = \frac{\text{volume of buffer in graduate} \times (0.004 - B)}{0.6}$$

where B is calculated from the equation:

$$B = \frac{1}{\text{antilog } (7.0 - \mathrm{pH})}\,.$$

Use the value of pH as determined above (6) for this calculation. The calculation is based on the assumption that the concentration of Na_2HPO_4 is less than $0.004M$. While the assumption is not necessarily true, calculations based on it will give amounts of the phosphate solution to be added to give a pH of 4.6. Solving the equation $\mathrm{pH} = pK + \log B/A$:

$$\operatorname{Log} B - \log A = \mathrm{pH} - pK$$
$$\operatorname{Log} B = \mathrm{pH} - pK + \log A$$

Changing signs, and substituting known values for pK and A,

$$\operatorname{Log} \frac{1}{B} = 6.3 - \mathrm{pH} - \log 0.2$$

$$\operatorname{Log} \frac{1}{B} = 6.3 - (-0{,}7) - \mathrm{pH} = 7.0 - \mathrm{pH}$$

$$\frac{1}{B} = \text{antilog } (7.0 - \mathrm{pH})$$

$$B = \frac{1}{\text{antilog } (7.0 - \mathrm{pH})} = \text{actual concentration of } Na_2HPO_4 \text{ now in the buffer.}$$

Now the amount of the Na_2HPO_4 solution to be added, designated by C, is equal to the number of millimoles needed, divided by 0.6, since 0.6 is the molarity of the stock Na_2HPO_4; and the number of millimoles needed is equal to the volume of buffer in the graduate × 0.004, minus this volume × B, its molarity. Or,

$$C = \frac{\text{volume of buffer in graduate} \times (0.004 - B)}{0.6} .$$

(*b*) If the pH is over 4.6, add an amount of the NaH_2PO_4 solution saved from step described above (3), calculated as follows (D = amount to be added):

$$D = \frac{\text{volume of buffer in graduate} \times (B - 0.004)}{0.004}$$

where B is calculated as above.

This calculation is based on the assumption that the concentration of Na_2HPO_4 is more than $0.004M$. The volume to which the buffer must be diluted with NaH_2PO_4 solution is equal to the number of millimoles of Na_2HPO_4 present divided by 0.004. And the number of millimoles of Na_2HPO_4 present is equal to the volume of buffer in the graduate × B. Further, the amount of NaH_2PO_4 solution to be added, designated by D, is equal to the final volume minus the volume already in the graduate. This gives:

$$D = \frac{\text{volume of buffer in graduate} \times (B - 0.004)}{0.004} .$$

8. Redetermine the pH, and if necessary, repeat step 7.

B. *Preparation of methylene blue stain for a 1 : 10,000 concentration:* A dropper was calibrated and found to drop 22 drops of $0.037M$ methylene blue solution per milliliter. The yeast solution to be counted was made by adding 0.10 g of yeast to 40 ml of buffer. The amount of dye needed to make the concentration 1 : 10,000 was calculated as follows:

Wt 40 ml water at 25°C	39.9 g
Wt yeast	0.1 g
Wt 7 drops dye	0.3 g
Total wt of suspension	40.3 g

$\frac{40.3}{10{,}000} = 0.00403$ g = amount of dye needed in yeast suspension. Therefore the dye solution must contain $22/7 \times 0.00403 = 0.01267$ g methylene blue per ml. $\frac{0.5}{0.01267} = 39.46$ = volume of dye solution containing 0.5 g methylene blue. Therefore, 0.5 g of methylene blue in 39.5 ml solution is equal to 1.27 g per 100 ml or 0.0127 g per ml. The molarity of this dye solution is then

$$\frac{0.0127 \times 1000}{\text{molecular wt methylene blue}} = \frac{12.7}{373.73} = 0.034M.$$

YEAST FERMENTATION AND POTASSIUM BROMATE AS FACTORS INFLUENCING THE HARMFUL EFFECTS OF WHEAT GERM ON BAKING QUALITY[1]

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It is well known that wheat germ has a harmful effect upon the baking characteristics of wheat flour, as indicated by the inferior handling properties of the dough and the production of small loaves possessing "green" or underfermented characteristics. Extension of the fermentation time, heat treatment, or water extraction of the germ and the addition of such oxidizing agents as potassium bromate to the dough decrease the deleterious effects. The literature on this subject has recently been reviewed and discussed by Sullivan, Howe, Schmalz, and Astleford (1940) and by Shen and Geddes (1942) and need not be detailed here.

It is sufficient to state that since the early studies reported by Geddes (1930) on the influence of wheat germ on baking quality, it has been established that a water-soluble reducing substance—glutathione—is the main factor responsible for its injurious baking effects. Moreover, it has been discovered that the flour proteinases, like papain, are inhibited by the common oxidizing agents which act as flour improvers, while they are activated by certain reducing agents such as sulfhydryl compounds and hydrogen sulfide. Some investigators believe that the improving action of oxidizing agents is due to their inhibitory effect on the flour proteases, while others hold that the reducing substances present in flour exert a direct action on the gluten proteins, since the effect of added glutathione on the physical properties of bread doughs is too rapid to be attributed solely to enzyme action. Moreover, the improvement noted in dough properties and bread characteristics by lengthening the fermentation time in the baking of germ-flour mixtures is incompatible with the theory that proteinase activation is primarily responsible for the harmful effects of wheat germ.

Recently Shen and Geddes (1942) determined the amino nitrogen and reducing matter contents of nonbromated and bromated doughs made from patent, fancy clear, and low-grade flours after varying fermentation times. These determinations were also carried out on similar doughs in which yeast activity was inhibited by the addition

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of octyl alcohol and an attempt was made to correlate these data with the baking behavior of the flours. With an increase in proteolytic activity and reducing matter content of the flours, a longer fermentation time and/or a higher bromate dosage was required to secure optimum dough handling properties and loaf characteristics. These supplementary baking effects of fermentation and bromate treatment could not readily be explained by the results of the amino nitrogen and reducing-matter determinations on either the fermenting or nonfermenting doughs. Thus, with few exceptions, the reducing-matter content of the basic doughs increased with fermentation, whereas it was decreased by the addition of bromate. Moreover, the amino nitrogen content of the nonfermenting doughs increased with increasing rest time. (In the fermenting doughs the amino nitrogen decreased with time of fermentation as a result of its utilization by the active yeast cells and hence could not be used as a measure of increased proteolysis with time.) The amino nitrogen contents of the bromated doughs were, however, lower than those of the corresponding nonbromated doughs, particularly in the presence of active yeast fermentation.

The supplementary baking effects of fermentation and bromate treatment noted by Geddes (1930) with germ-flour mixtures and by Shen and Geddes (1942), particularly with the fancy clear and low-grade flours, naturally suggested that the harmful effects of germ might be significantly reduced by fermenting it with yeast in the presence of oxidizing agents before mixing with the flour and other dough ingredients. The experiments reported here were accordingly undertaken to investigate the effects of various pretreatments of wheat germ on its baking properties and to follow the changes in amino nitrogen and reducing-matter content resulting from these treatments. It appeared that such a study might throw further light on the relative importance of proteolytic activity and reducing matter content in relation to flour improvement by oxidizing agents.

During the course of these experiments Hullett (1940) published a brief note describing a method for treating wheat germ so that when it is incorporated with the flour the adverse effects normally associated with its presence are largely avoided. This method involves prefermenting the germ for a sufficient length of time to oxidize the glutathione, as indicated by failure to give the nitroprusside reaction. The germ sponge is then mixed with the other dough ingredients and baked in the usual manner. With the use of 4% raw wheat germ (on a flour basis) the bread made by this method was very similar to ordinary white bread; with 10% germ, the volume and texture were only slightly affected but the crumb was light brown in color. From

these observations a commercial process was devised which made it possible to produce an acceptable germ loaf containing up to 10% of wheat germ on a flour basis.

Hullett also noted that improved baking results were obtained by prefermenting the low-grade portions of long-extraction flours and suggested that glutathione elimination by yeast may play a significant part in the ordinary dough ripening process. In a later paper published after the completion of the present study, Hullett and Stern (1941) reported the experiments carried out in developing the commercial process. The elimination of reduced glutathione, as indicated by a negative nitroprusside test, was associated with the fermenting activity of the yeast, since iodoacetic acid, sodium fluoride, and acetone, which inhibited fermentation, also prevented glutathione destruction. The destruction of glutathione apparently is connected with an enzyme mechanism effective in the raw germ, since destruction did not take place when boiled germ was fermented; moreover, destruction took place when glutathione was added to unboiled germ ferments, but not when it was added to a boiled germ ferment or to a fermenting sugar solution. These workers report that the disappearance of the glutathione reaction as a result of fermentation cannot be ascribed to oxidation to the S-S form since treatment with various reducing agents failed to restore the nitroprusside test. In view of these observations they express doubt that the action of potassium bromate in a bread dough is actually an oxidizing one. It was also observed that germ fermentation not only eliminated the glutathione in the germ but also in the yeast and that a brown to pink color began to develop on the surface of the ferment as soon as a negative nitroprusside reaction was obtained.

Experimental

Materials and methods: The experimental materials consisted of a highly refined untreated second middlings flour (12.5% protein, 0.36% ash, 13.5% moisture basis) and commercial wheat germ (27.3% crude protein, 9.9% petroleum ether extract, 13.5% moisture basis), freshly milled from hard red spring wheat. The germ, which contained 10.4% moisture, was ground in a Wiley laboratory mill to pass the 0.5-mm sieve and stored, along with the flour, at approximately 3°C when not in use.

In the major series of experiments, baking tests were conducted by the A. A. C. C. basic method (as outlined in *Cereal Laboratory Methods*, 4th ed., 1941) and also by a bromate method (basic formula +0.001% $KBrO_3$) on the middlings flour alone and with 5% and 10% of the flour replaced by wheat germ, using dough fermentation times

of 1.5, 3.0, and 4.5 hours. The germ used in these tests was submitted to the following treatments: (1) control—germ added directly to the flour, and (2) pretreated by allowing to stand or ferment in aqueous suspension for 1.5, 3.0, 4.5, 6.0, and 16 hours, respectively, with the following additions: nil, potassium bromate, yeast, and yeast plus potassium bromate. The control series represents the regular basic and bromate straight-dough experimental baking test, while the germ pretreatments involve variations of a sponge and dough baking method in which part of the water, plus the potassium bromate and/or the yeast as specified above, is included in the sponge.

In making up the germ sponges 2 ml of water per gram of germ was used, since it yielded a mixture from which the germ did not settle out. At the end of the sponge time the remaining liquid and the regular ingredients not added to the sponge were mixed in the usual manner to form the dough. In all cases where wheat germ was present it replaced an equivalent dry-matter weight of flour. Three hundred grams of flour or flour and germ and proportional quantities of the other ingredients were mixed in a Hobart-Swanson mixer for two minutes and three 150-g portions scaled off, one for each of the three fermentation times. The punching and molding schedules for the various dough fermentations and other details of the baking method were the same as those described by Shen and Geddes (1942).

In the experiments outlined above only nil and 1 mg $KBrO_3$ per 100 g of germ and flour were used. A similar series of experiments was carried out with a germ pretreatment time of 3.0 hours and bromate levels of 0, 1, 2, 4, and 6 mg of $KBrO_3$ with 5% germ and 0, 2, 4, 6 and 10 mg of $KBrO_3$ with 10% germ.

Baking tests were also conducted in which 10%, 15%, and 20% of the flour was replaced by wheat starch, untreated and pretreated germ, respectively. The wheat starch was prepared in the laboratory from hard red spring wheat flour. The "pretreated" germ required for each loaf was fermented for three hours with 3 g of yeast and 1 mg of $KBrO_3$.

In another experiment, wheat germ was continuously extracted with petroleum ether for 24 hours and the extracted germ, after allowing the solvent to evaporate, employed in baking tests in comparison with unextracted germ. In these tests the original and extracted germ samples were used in 5%, 10%, and 15% levels and in each case the germ was employed in the straight-dough baking method and also in a sponge and dough procedure in which the germ required per loaf was prefermented 3 hours in a sponge with 3 g of yeast.

It must be emphasized that the sponge treatments involving yeast and potassium bromate are not directly comparable for differing germ

TABLE I

MEAN LOAF VOLUME DATA FOR PATENT FLOUR AND 5% GERM-FLOUR MIXTURES CONTAINING GERM SUBJECTED TO VARIOUS TREATMENTS

Dough fermentation	Patent flour	Loaf-volume data for germ-flour mixtures, with germ pretreated for various periods:					
		0 hr	1.5 hrs	3 hrs	4.5 hrs	6 hrs	16 hrs
hrs	*cc*	*cc*	*cc*	*cc*	*cc*	*cc*	*cc*
	Basic	GERM SUSPENSION					
1.5	710	540	530	555	530	530	570
3.0	650	555	520	600	570	550	640
4.5	600	560	555	565	620	570	610
	1 mg BrO_3	GERM SUSPENSION + 1 MG BROMATE					
1.5	705	565	520	560	575	540	615
3.0	690	615	570	560	580	565	685
4.5	615	650	540	580	570	545	690
	1 mg BrO_3	GERM SUSPENSION (1 MG BROMATE IN DOUGH)					
1.5	705	565	525	550	555	560	635
3.0	690	615	610	640	655	625	685
4.5	615	650	640	645	660	630	635
	Basic	GERM SUSPENSION + YEAST					
1.5	710	540	560	675	670	730	705
3.0	650	555	600	665	690	710	710
4.5	600	560	585	620	625	680	580
	1 mg BrO_3	GERM SUSPENSION + 1 MG BROMATE + YEAST					
1.5	705	565	630	670	710	705	695
3.0	690	615	655	690	690	685	650
4.5	615	650	630	645	640	645	630
	1 mg BrO_3	GERM SUSPENSION + YEAST (1 MG BROMATE IN DOUGH)					
1.5	705	565	605	680	705	705	—
3.0	690	615	705	675	660	655	—
4.5	615	650	620	655	580	535	—

percentages incorporated with the flour at the dough stage. In order to obtain a similar yeast and bromate concentration in the dough, a constant quantity of each had to be used in the sponge; thus with 5% germ-flour loaves, the ratio of germ to yeast in the pretreatment was 5 : 3, whereas with the 10% germ-flour loaves the ratio was 10 : 3.

TABLE II

MEAN LOAF VOLUME DATA FOR PATENT FLOUR AND 10% GERM-FLOUR MIXTURES CONTAINING GERM SUBJECTED TO VARIOUS TREATMENTS

Dough fermentation	Patent flour	Loaf-volume data for germ-flour mixtures, with germ pretreated for various periods:					
		0 hr	1.5 hrs	3 hrs	4.5 hrs	6 hrs	16 hrs
hrs	*cc*	*cc*	*cc*	*cc*	*cc*	*cc*	*cc*
	Basic	GERM SUSPENSION					
1.5	710	500	490	525	525	485	570
3.0	650	490	500	510	500	490	580
4.5	600	520	500	510	500	485	485
	1 mg BrO_3	GERM SUSPENSION + 1 MG BROMATE					
1.5	705	520	470	515	530	510	535
3.0	690	555	530	525	515	510	570
4.5	615	580	515	525	510	505	465
	1 mg BrO_3	GERM SUSPENSION (1 MG BROMATE IN DOUGH)					
1.5	705	520	495	520	530	530	570
3.0	690	555	515	530	540	535	610
4.5	615	580	520	525	545	510	500
	Basic	GERM SUSPENSION + YEAST					
1.5	710	500	520	575	570	600	600
3.0	650	490	520	565	535	560	585
4.5	600	520	480	490	480	500	435
	1 mg BrO_3	GERM SUSPENSION + 1 MG BROMATE + YEAST					
1.5	705	520	535	585	610	595	600
3.0	690	555	545	590	575	560	565
4.5	615	580	485	520	510	490	390
	1 mg BrO_3	GERM SUSPENSION + YEAST (1 MG BROMATE IN DOUGH)					
1.5	705	520	535	605	595	615	590
3.0	690	555	565	570	550	575	590
4.5	615	580	490	490	510	505	455

Amino nitrogen and reducing matter content were determined upon extracts of treated and untreated wheat germ, employing the methods described by Shen and Geddes (1942). Portions of each of the extracts were tested for sulfhydryl groups by the nitroprusside test.

Determinations of the pH of some of the germ suspensions were made employing a glass electrode.

Baking results: The mean loaf volume data for the patent flours and the 5% and 10% germ-flour mixtures involving various germ treatments are recorded in Tables I to IV; the loaf volumes for the 5% germ treatments are also diagrammatically represented in Figures 1 and 2. While the handling properties of the doughs were noted at various stages of the fermentation and the loaves judged for external appearance, crumb grain, texture, and crumb color, these data have not been tabulated here since they are closely related to loaf volume. Some idea of the general loaf characteristics for certain of the germ treatments may be gained from the photographs reproduced in Figures 3 and 4.

TABLE III

MEAN LOAF VOLUME DATA FOR PATENT FLOUR AND 5% GERM-FLOUR MIXTURES SHOWING EFFECTS OF PREFERMENTATION FOR THREE HOURS AND VARIOUS BROMATE DOSAGES

Dough fermentation	Loaf volume for patent flour		Loaf volumes for germ-flour mixtures with various dosages of $KBrO_3$ in mg:[1]				
	Basic	1 mg $KBrO_3$	0	1	2	4	6
hrs	*cc*	*cc*	*cc*	*cc*	*cc*	*cc*	*cc*
			GERM SUSPENSION WITH BROMATE				
1.5	710	705	560	560	565	620	645
3.0	650	695	600	565	595	725	710
4.5	600	615	565	580	575	675	645
			GERM SUSPENSION (BROMATE IN DOUGH)				
1.5	710	705	560	550	630	700	740
3.0	650	695	600	640	715	920	660
4.5	600	615	565	665	670	520	450
			GERM SUSPENSION + YEAST WITH BROMATE				
1.5	710	705	675	675	740	690	—
3.0	650	695	665	690	725	620	—
4.5	600	615	620	650	665	570	—
			GERM SUSPENSION + YEAST (BROMATE IN DOUGH)				
1.5	710	705	675	680	740	770	—
3.0	650	695	665	675	655	590	—
4.5	600	615	620	655	565	455	—

[1] The germ pretreatment consisted of standing in aqueous suspension for three hours with and without yeast and/or potassium bromate as indicated.

TABLE IV

MEAN LOAF VOLUME DATA FOR PATENT FLOUR AND 10% GERM-FLOUR MIXTURES SHOWING EFFECTS OF PREFERMENTATION FOR THREE HOURS AND VARIOUS BROMATE DOSAGES

Dough fermentation	Loaf volume for patent flour		Loaf volumes for germ-flour mixtures with various dosages of $KBrO_3$ in mg:[1]				
	Basic	1 mg $KBrO_3$	0	2	4	6	10
hrs	*cc*	*cc*	*cc*	*cc*	*cc*	*cc*	*cc*
			GERM SUSPENSION WITH BROMATE				
1.5	710	705	525	520	530	520	535
3.0	650	695	525	545	560	545	590
4.5	600	615	510	510	535	530	565
			GERM SUSPENSION (BROMATE IN DOUGH)				
1.5	710	705	525	520	610	625[2]	610[2]
3.0	650	605	525	560	625	500	515
4.5	600	615	510	550	595	455	410
			GERM SUSPENSION + YEAST WITH BROMATE				
1.5	710	705	575	585	—	610[2]	—
3.0	650	695	565	590	—	550	—
4.5	600	615	490	520	—	440	—
			GERM SUSPENSION + YEAST (BROMATE IN DOUGH)				
1.5	630[2]	670[2]	575	605	700[2]	675[2]	670[2]
3.0	675	700	565	570	510	495	420
4.5	575	665	490	490	380	370	350

[1] The germ pretreatment consisted of standing in aqueous suspension for three hours with and without yeast and/or potassium bromate as indicated.

[2] Baking tests in this column conducted with another sample of patent flour obtained from the same source.

When the germ was added directly to the flour and baked by the basic formula, the doughs were soft and sticky upon removal from the mixer, particularly those containing 10% of wheat germ. As fermentation progressed, the handling properties improved. The loaves baked from the basic doughs after only 1.5 hours of fermentation possessed pronounced "green" or underfermented characteristics. They were small in volume, had sharp corners, a glossy side crust with small round holes, a reddish top crust, flat top and no break; the cells of the crumb were large and round with thick cell walls. As the fermentation was extended to 4.5 hours, the dough-handling properties improved and the loaves were somewhat larger in volume and had less pronounced underfermented characteristics.

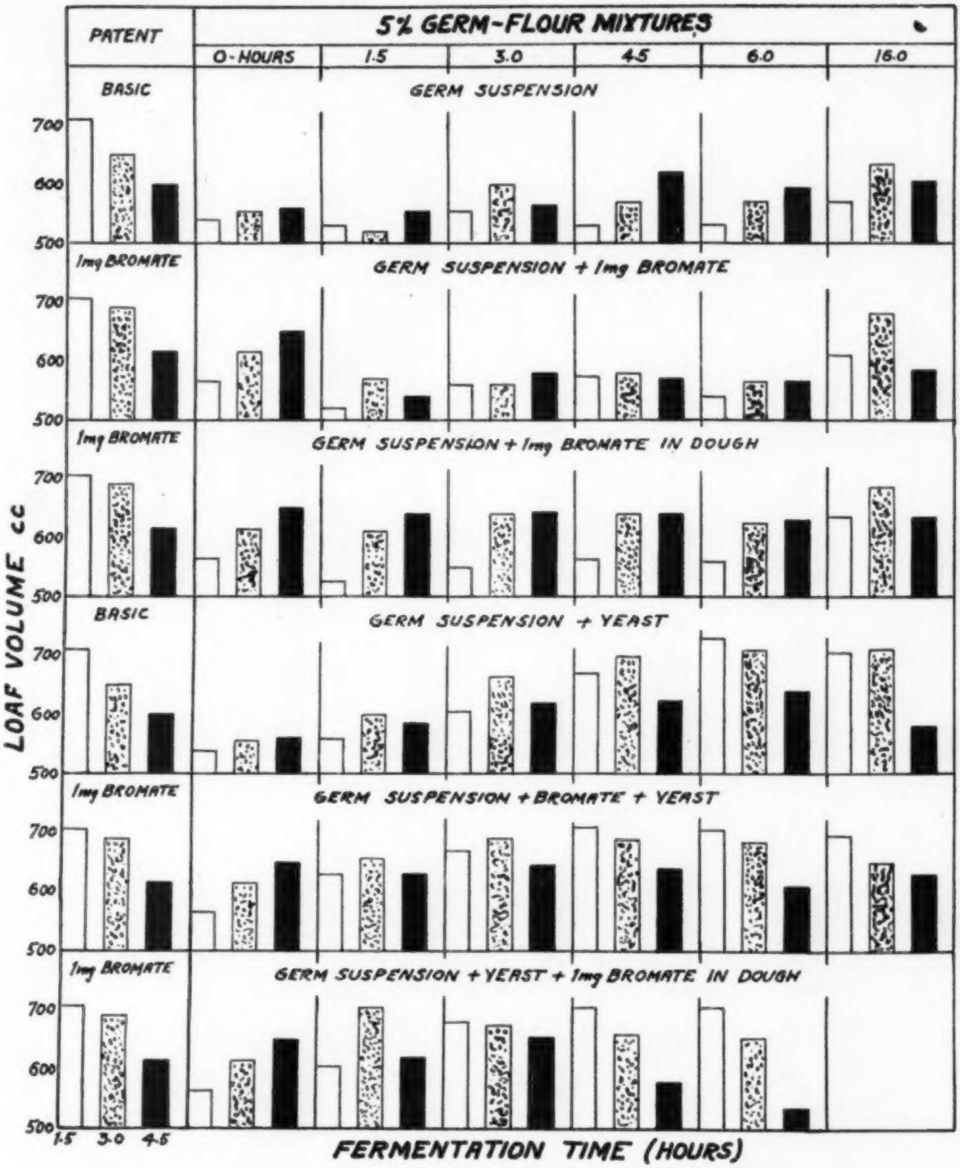

Fig. 1. Effect of wheat germ on the loaf volume of 5% germ-flour mixtures at dough fermentation times of 1.5, 3.0, and 4.5 hours. Diagrams show the effects of adding untreated germ at the dough stage (0 hours) as compared with germ added after various sponge treatments, including standing in aqueous suspension (with and without $KBrO_3$) and yeast fermentation (with and without $KBrO_3$) for various times.

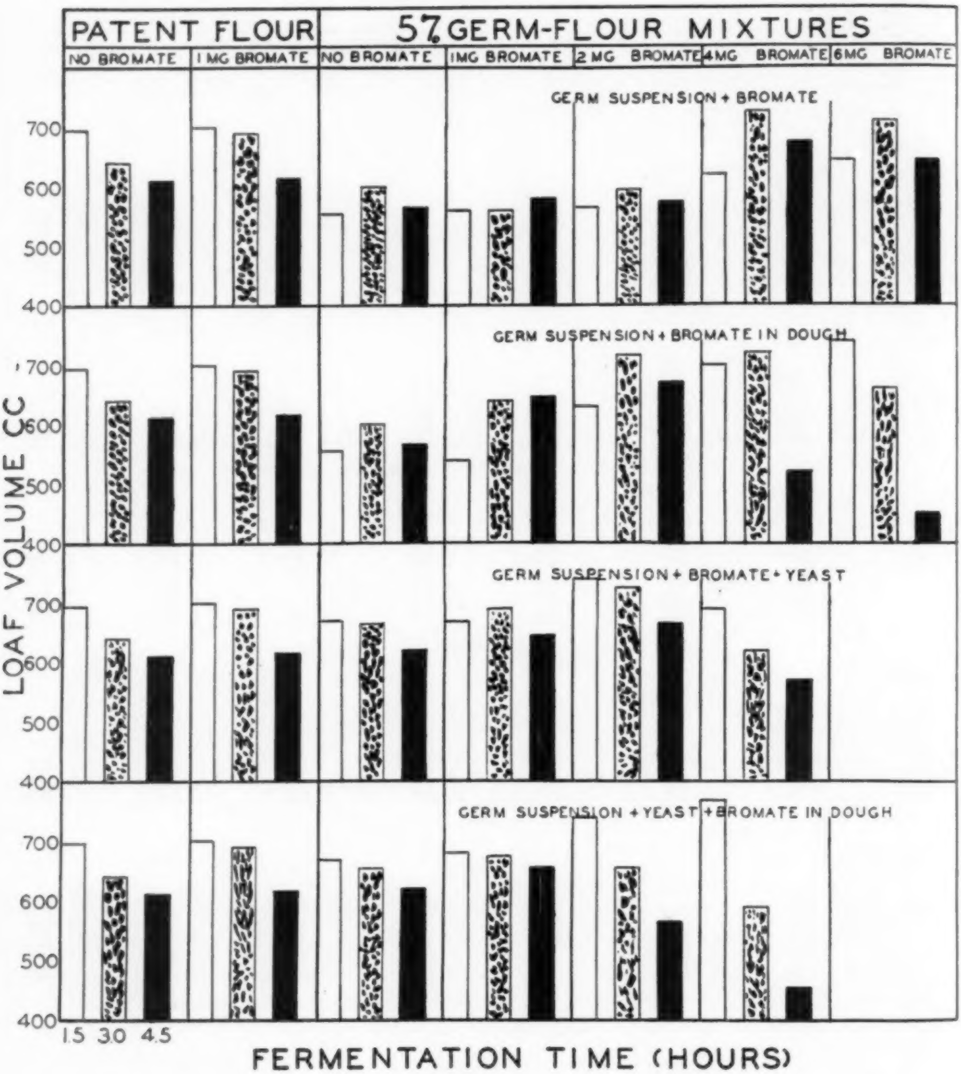

Fig. 2. Effect of wheat germ on the loaf volume of 5% germ-flour mixtures at dough fermentation times of 1.5, 3.0, and 4.5 hours. Diagrams show the effects of adding wheat germ at the dough stage after standing or fermenting for 3 hours with additions of 0, 1, 2, 4 and 6 mg of $KBrO_3$ per 5 g of germ.

The inclusion of potassium bromate in the baking formula, however, had a very pronounced improving effect when the germ-flour mixtures were baked by the regular straight-dough experimental procedure. The physical properties of the doughs were enhanced shortly after mixing and during the early part of the fermentation but doughs with the highest bromate treatments became "tough" as fermentation

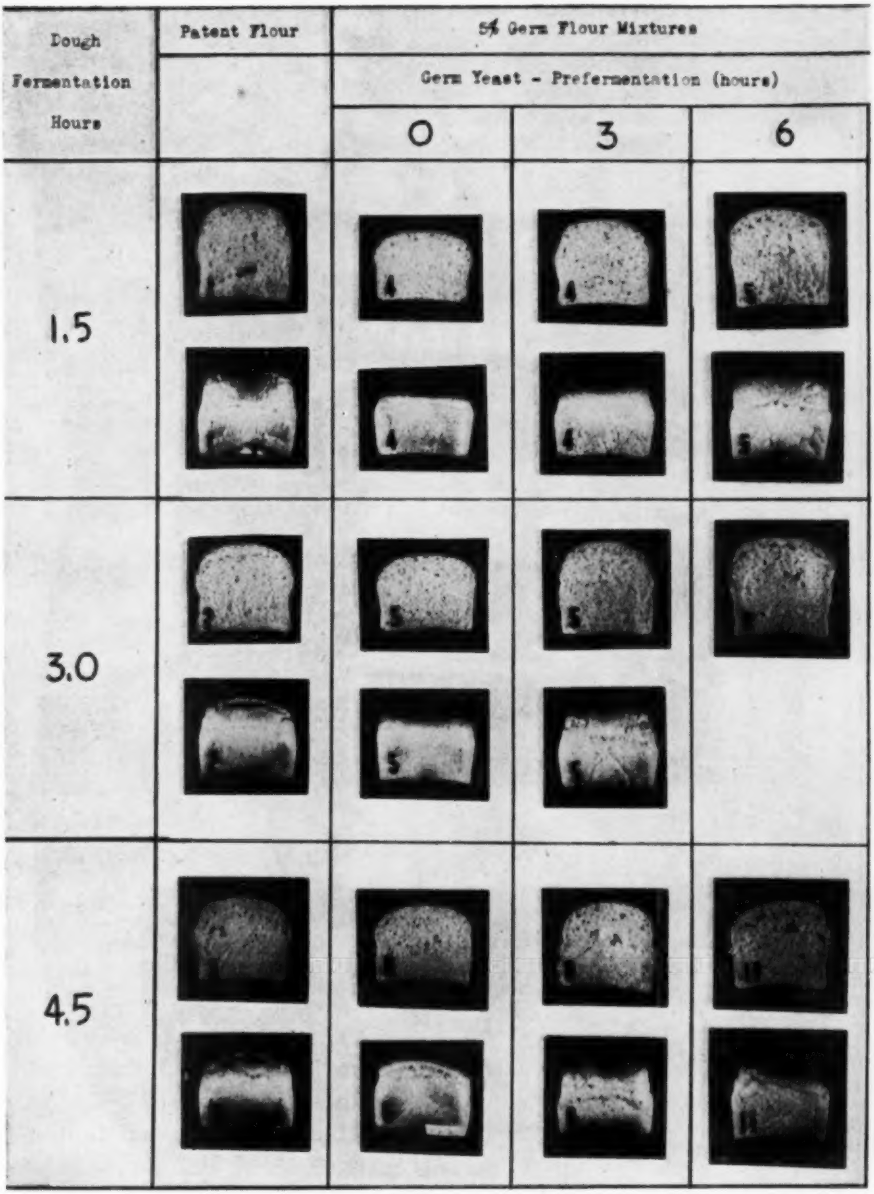

Fig. 3. Photographs of loaves baked by basic formula showing the effect of prefermenting germ for various times on the baking properties of 5% germ-flour mixtures.

was extended. This "over effect" was more noticeable with the 10% than with the 5% germ flour doughs. These progressive changes in dough properties were paralleled by changes in the bread character-

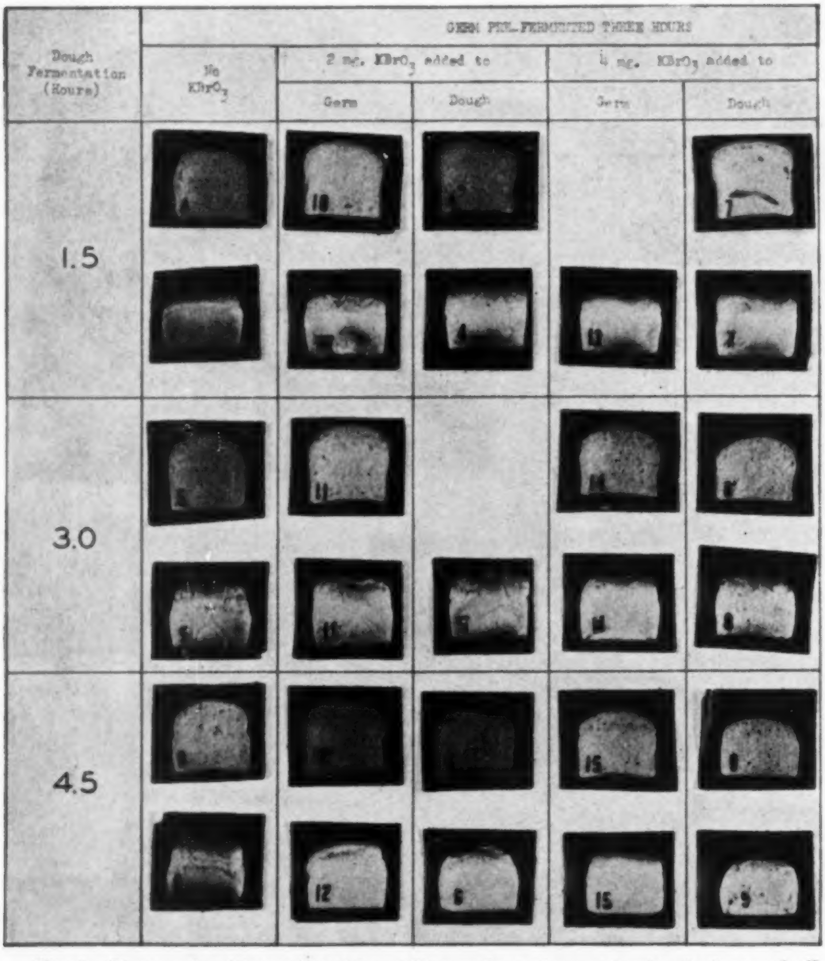

Fig. 4. Photographs of loaves baked from 5% germ-flour mixtures showing the increased efficiency of potassium bromate when added at the dough stage. For all loaves the germ was prefermented 3 hours; those in the left-hand column were baked by the basic formula, while those in the remaining columns were baked with the addition of 2 or 4 mg of $KBrO_3$ to the germ sponge or dough, as indicated.

istics. The underfermented characteristics became less pronounced and loaf volume increased to an optimum and then decreased. As the bromate dosage was increased, the optimum loaf, in general, was obtained at shorter dough fermentation times. The highest bromate dosages of 6 and 10 mg per loaf in the 5% and 10% germ-flour mixtures, respectively, gave loaves possessing slightly "old" characteristics at 4.5 hours of fermentation.

Turning now to the effects of pretreatment, allowing the germ to stand in aqueous suspension for varying periods of time up to and including 6 hours had little influence on its baking effects. A slight

improvement in both the 5% and 10% germ-flour loaves was noted for the 16-hour treatment. In these instances there was evidence of some fermentation, presumably by wild yeasts. The addition of 1 mg of $KBrO_3$ to the aqueous germ suspension likewise had little effect but, as shown in Tables III and IV and Figures 2 and 4, large dosages (4 and 6 mg in the 5% germ series and 10 mg in the 10% germ series), somewhat decreased the deleterious effects of the germ.

Prefermentation of the germ, however, strikingly reduced its harmful baking effects, the improvement in this regard being supplemented by the presence of potassium bromate in the fermenting germ sponge. The influence of potassium bromate was markedly greater when it was incorporated at the dough stage; in this instance the larger bromate dosages gave doughs that were "short" and "dead" and loaves possessing pronounced overfermented characteristics when the dough fermentation was extended beyond 1.5 hours and especially for the longer germ prefermentation times. These data clearly show that

TABLE V

MEAN LOAF VOLUME DATA BY BASIC FORMULA SHOWING EFFECTS OF CORRESPONDING ADDITIONS OF WHEAT STARCH, UNTREATED GERM, AND PREFERMENTED-BROMATE-TREATED GERM[1]

Flour diluent	Loaf volumes with dough fermentation times in hours: 1.5 hrs	3.0 hrs	4.5 hrs
	cc	*cc*	*cc*
10% DILUENT ADDED			
Wheat starch	625	625	580
Untreated germ	500	490	520
Pretreated germ	585	585	520
15% DILUENT ADDED			
Wheat starch	560	565	570
Untreated germ	470	460	480
Pretreated germ	495	490	400
20% DILUENT ADDED			
Wheat starch	535	535	545
Untreated germ	370	370	380
Pretreated germ	395	335	285

[1] The required quantities of germ per loaf were prefermented 3 hours with 3 grams yeast and 1 mg of $KBrO_3$.

the prefermentation of the germ shortens the fermentation required in the dough stage to secure optimum baking results and that potassium bromate is much more effective in reducing the deleterious baking effects of wheat germ when it is added to the dough. It is of interest to note that under optimum conditions the loaf volumes of the germ-flour doughs exceeded that of the patent flour alone; aside from crumb color, the other bread characteristics were eminently satisfactory.

The crumb color of these optimum loaves was greyish-yellow. Loaves representative of undertreatment of the germ possessed a yellowish-brown crumb, while those in which the germ was overtreated exhibited a distinct reddish-brown crumb, the intensity of the reddish cast being more or less proportional to the extent of the overtreatment as indicated by the decrease in loaf volume from the optimum. The surface of the fermenting germ suspensions also developed a brown color with time.

The loaf-volume data recorded in Table V for patent flour diluted with 10%, 15%, and 20% wheat starch, untreated wheat germ, and

TABLE VI

MEAN LOAF VOLUME DATA FOR 5%, 10%, AND 15% GERM-FLOUR MIXTURES SHOWING EFFECTS OF PREFERMENTATION ON UNTREATED AND PETROLEUM-ETHER-EXTRACTED GERM

Dough fermentation time	Loaf volume					
	Unextracted germ, germ level in %:			Petroleum-ether-extracted germ, germ level in %:		
	5%	10%	15%	5%	10%	15%
hrs	*cc*	*cc*	*cc*	*cc*	*cc*	*cc*
GERM ADDED DIRECT TO DOUGH						
1.5	540	500	470	550	530	—
3.0	555	490	460	580	520	—
4.5	560	525	480	565	490	—
GERM PREFERMENTED 3.0 HOURS						
1.5	675	575	495	680	565	410
3.0	640	565	490	665	535	365
4.5	620	490	400	625	440	315

fermented wheat germ (3.0 hours with 1 mg $KBrO_3$ present), respectively, show quite clearly that the harmful baking effects of the germ are not due merely to a dilution effect. Moreover, these data indicate that prefermentation of the germ is relatively ineffective with germ levels exceeding 10%. That this decrease in the relative efficiency of prefermentation with large germ concentrations is not due primarily to the presence of larger amounts of lipids in the dough, which *per se* have a harmful effect on gluten, is shown by the comparative loaf-volume data for 5%, 10%, and 15% germ-flour loaves (Table VI) containing unextracted germ and petroleum-ether-extracted germ, with and without prefermentation. The responses of the 5% germ-flour loaves made from unextracted and extracted germ to prefer-

mentation, respectively, are virtually identical while the 10% and 15% germ-flour series show larger volumes and greater responses to prefermentation in the instance of the unextracted germ.

Physico-chemical and chemical tests: In our hands the nitroprusside reaction did not prove to be particularly sensitive. Patent flour extracts gave a negative test and untreated germ extracts gave definitely positive tests; on the other hand extracts of 5% germ-flour mixture gave an indecisive test. When wheat germ stood in aqueous suspension with or without the addition of potassium bromate strong positive tests were obtained; prefermentation, however, progressively decreased the intensity of the color produced, but it was impossible to determine with any degree of certainty the fermentation time required to secure a negative test or whether the inclusion of potassium bromate in the fermenting germ sponge decreased this time. It must be pointed out, however, that no particular effort was directed to perfecting the technique of this qualitative test since quantitative determinations of reducing matter content were also being carried out.

The results of pH determinations on nonfermenting and fermenting germ suspensions, with and without the addition of potassium bromate, are recorded in Table VII. Wheat germ has a relatively high

TABLE VII

EFFECT OF FERMENTATION AND POTASSIUM BROMATE ON pH OF WHEAT GERM SUSPENSIONS

Nature of suspension	Time of standing in hours: 0 hrs	1.5 hrs	3.0 hrs
	pH	*pH*	*pH*
Aqueous germ suspension	6.4	6.4	6.6
Aqueous germ suspension + 2 mg $KBrO_3$ per 5 g	6.6	6.5	6.5
Aqueous germ suspension + 3 g yeast per 5 g germ	6.1	6.0	6.2
Aqueous germ suspension + 3 g yeast + 2 mg $KBrO_3$ per 5 g	6.1	5.9	6.0

buffer capacity and the changes in acidity due to fermentation of the germ appear entirely too small to account for the marked improving effect of prefermentation on the baking properties.

Because of lack of experimental material, only a limited number of tests for the effect of germ treatment on proteinase activity and reducing-matter content was carried out. The mean results for extracts prepared from nonfermenting and fermenting germ suspensions with and without bromate after various time intervals are given in Table VIII. Without yeast or bromate the amino nitrogen and reducing-matter content increased with time of standing, the extent of the increase being much greater for the latter. The inclusion of 4

mg of $KBrO_3$ in the suspension tended to inhibit proteolysis and the increase in reducing-matter content with time.

When fermentation took place, the amino nitrogen compounds were utilized by the yeast, and at three hours of fermentation time the amino nitrogen was lower than in the original germ or in a nonfermenting suspension allowed to stand for the same length of time. Yeast

TABLE VIII

EFFECT OF GERM PRETREATMENT ON AMINO NITROGEN AND REDUCING MATTER CONTENT

Treatment time *hrs*	Aqueous suspensions— $KBrO_3$ in mg:			Yeast suspensions— $KBrO_3$ in mg:	
	0 mg	2 mg	4 mg	0 mg	4 mg
AMINO NITROGEN CONTENT IN MG PER G OF GERM					
0	2.0	2.2	2.0	—	—
1.5	2.1	—	—	1.3	—
3.0	2.2	—	2.0	1.5	1.3
4.5	2.6	—	—	1.6	—
5.0	2.4	—	—	—	—
REDUCING MATTER CONTENT ML $0.01N$ IODINE PER G GERM[1]					
0	1.3	1.3	1.2	—	—
1.5	1.9	—	—	1.6	—
3.0	2.6	—	1.9	1.5	1.0
4.5	2.5	—	—	1.5	—
5.0	2.9	—	—	—	—

[1] Positive nitroprusside tests were obtained with the aqueous germ suspensions, the intensity of the test being reduced when potassium bromate was present. The yeast-germ suspensions gave either negative or doubtful tests at times corresponding to those at which reducing matter content was determined.

fermentation, moreover, greatly inhibited the increase in reducing-matter content, which was observed with time of standing in the instance of the nonfermenting suspensions; the inclusion of 4 mg of $KBrO_3$ in the fermenting suspension further lowered the reducing-matter content. However, both fermentation and the presence of potassium bromate were required to keep the reducing-matter content at approximately the original level of a freshly made suspension. Yet the germ suspension, fermented for three hours, gave a negative nitroprusside test, whereas the original germ gave a strong positive test.

Discussion

The baking results demonstrate that prefermentation of wheat germ, as first announced by Hullett (1940) and Hullett and Stern (1942), markedly lowers its deleterious effect upon flour baking quality.

Such pretreatment, however, is not particularly helpful when germ levels of 10% and higher are employed. That this decrease in baking response to pretreatment with high germ levels is apparently not to be ascribed to masking by the harmful effects of the germ lipids is shown by a similar lack of response in the instance of prefermented petroleum-ether-extracted germ when used at correspondingly high levels.

An outstanding feature of the results is the difference in the efficiency of potassium bromate as an improver when added to aqueous germ suspensions, fermenting germ suspensions, and to the fermenting germ-flour doughs, respectively. Baker and Mize (1939) have previously shown that bromate has little effect in the absence of both mechanical action and yeast fermentation. When added to the nonfermenting germ suspensions very large dosages were required to effect an improvement in dough handling properties and in general bread quality. The efficiency was considerably increased by adding yeast to the germ sponge but it was much more effective when added at the dough stage. Long prefermentation of germ coupled with large bromate dosages in the doughs resulted in an "excess bromate effect" as shown by bread possessing pronounced overfermented characteristics, particularly with long dough fermentation times. Moreover the "excess effect" appeared to be more pronounced with bread containing more germ. While it is known that the rate of oxidation by bromate increases with an increase in acidity, the changes in pH observed in these studies seem entirely too small to explain the marked differences observed. A complete theory of the nature of bromate action must obviously provide an explanation of the supplementary effects of yeast fermentation and bromate efficiency.

Hullett and Stern (1942) ascribe the beneficial effects of prefermentation to glutathione "destruction," as indicated by their failure to obtain a positive nitroprusside test when the germ was prefermented for a sufficient length of time to secure maximum improvement. In the present studies, similar observations were made but the qualitative indications were not in accord with the quantitative results for reducing matter content, as determined by the Freilich (1941) procedure. On the other hand the reducing-matter values were not correlated with the baking behavior. Thus the reducing-matter content of aqueous germ suspensions increased with time of standing but this was not accompanied by any significant change in the loaf characteristics of flour-germ admixtures in which the germ component had been allowed to stand in aqueous suspension for varying times. Prefermentation, especially when potassium bromate was present, tended to inhibit the increase, but extensive pretreatment, which had

marked baking effects, was necessary to maintain the reducing matter (and amino nitrogen) content at the level present in the original untreated germ.

These observations naturally raise several questions which cannot be answered on the basis of the present studies. What specific substances are being measured by the iodine titration procedure and which of these are of significance in relation to baking behavior? Will a modification of the conditions of the test or other quantitative techniques give more significant results? Tests conducted on the fermenting germ-flour doughs which were not reported in detail in this paper showed that, as observed by Shen and Geddes (1942) in the instance of doughs made from fancy clear and low-grade flours, the reducing-matter content tended to increase with fermentation, the increase being inhibited by bromate. This suggests that S-S linkages in the proteins are being converted to SH groups and that the oxidation of the latter by bromate may have a profound effect upon gluten properties.

The uncertainties regarding the significance of the reducing-matter values and the limited amount of chemical data render it inadvisable to reach any conclusions as to the relative importance of protease activity and reducing-matter content in relation to the improving effect of fermentation and potassium bromate additions. This is further complicated by the fact that, in the presence of yeast fermentation, amino nitrogen loses its significance as an index of proteolytic activity. However, a few tests on octyl-alcohol-treated doughs left no doubt that the addition of germ to flour markedly increased the proteolytic activity, the extent of the increase being less in the presence of potassium bromate. Since, however, an increase in proteolysis gave improved baking results with untreated germ, the improving effect of potassium bromate cannot be ascribed entirely to protease inhibition; if this were the case, continued improvement without any excess effect should be observed with increasing bromate dosages and at the shorter fermentation times. Moreover, one would expect the greatest improving action of bromate to occur when it is added directly to the germ by decreasing the reduced glutathione content and thereby more or less inactivating the germ proteases before they are added to the flour.

The fact that bromate is a more efficient flour improver when added to the fermenting dough rather than to the fermenting sponge, despite the lower effective bromate concentration, suggests that it exerts a direct action on the gluten proteins.

Summary

Baking tests conducted by the A. A. C. C. basic formula on mixtures of varying percentages of commercial wheat germ with a highly refined hard red spring wheat middlings flour using fermentation times of 1.5, 3.0 and 4.5 hours showed that the germ had a marked deleterious effect upon baking quality. The addition of wheat germ to the flour resulted in soft, sticky doughs and the production of loaves of small volume possessing "green" or underfermented characteristics. Dough-handling qualities and loaf characteristics improved as the fermentation time was extended from 1.5 to 4.5 hours. The inclusion of potassium bromate in the baking formula markedly improved dough handling properties and loaf characteristics.

Allowing the germ to stand in aqueous suspension for periods up to 6 hours prior to mixing with the flour had little or no improving action but when potassium bromate was present in the aqueous suspension in relatively large concentrations (4 mg per 5 g of germ) some reduction in the harmful effects of germ on the baking characteristics of 5% and 10% germ-flour mixtures was noted.

Prefermentation of the germ with yeast progressively decreased the harmful effects with time of prefermentation up to about 4.5 hours with 5% germ-flour doughs. Longer prefermentation times gave little further improvement. The presence of potassium bromate in the fermenting germ suspension further improved the baking behavior of the germ-flour mixtures at short dough fermentation periods. Overeffects, resulting in the production of bread with "old" or overfermented characteristics, were obtained with long germ prefermentation periods and/or long dough fermentation periods, particularly when potassium bromate was added at the dough stage.

Under optimum conditions, bread baked from 5% and 10% germ-flour mixtures approached the quality of that baked from the patent flour alone, with the exception of crumb color. Overtreatment of the germ resulted in bread possessing a brownish red crumb.

The improving action due to germ prefermentation and/or potassium bromate was most pronounced in the 5% germ-flour series. These treatments were relatively ineffective with 15% and 20% germ-flour mixtures. Studies with petroleum-ether-extracted germ showed that failure to obtain improvement with the higher germ levels was not due to the larger quantities of germ lipids present in the doughs.

The efficiency of potassium bromate as a flour improver was greatest when used at the dough stage and least when added to aqueous germ suspensions. In the fermenting germ sponges relatively slight

decreases in pH occurred, which seemed insufficient to explain the greater efficiency of bromate in the presence of yeast fermentation.

In aqueous germ suspensions, amino nitrogen and reducing matter content, particularly the latter, increased with time of standing. The presence of 4 mg of potassium bromate per 5 g of germ in the aqueous suspension tended to inhibit proteolysis and the increase in reducing-matter content with time.

In fermenting germ suspensions, the amino nitrogen compounds were utilized by the yeast. Yeast fermentation, particularly in the presence of potassium bromate, greatly inhibited the increase in reducing-matter content which was observed with time in the instance of nonfermenting suspensions.

Prefermentation for a sufficient time resulted in a negative nitroprusside test, despite quantitative levels of reducing matter equal to or exceeding that of the original germ which gave a strong positive nitroprusside test. The reducing-matter values were not correlated with baking behavior.

The much greater efficiency of potassium bromate when added to fermenting germ-flour dough rather than to fermenting germ sponge, despite a lower effective concentration, suggests that it exerts a direct action on the gluten proteins.

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THE DETERMINATION OF FLOUR PARTICLE SIZE [1]

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Measurements of particle size have been made on materials ranging from mine-run coal and other minerals to carbon black and rouge for glass polishing. The methods have been diverse, and frequently a method adequate for one type of material has proved totally unsatisfactory for another. In consequence the literature on particle-size measurement is voluminous and in many cases not pertinent to measurements on flour. Brief mention will be made, however, of various types of measurements which have been employed in the past and indications of their applicabilities for flour studies will be given.

Sieving is the method that first suggests itself. In this procedure the weight of material that passes a screen of one size and fails to pass a screen of smaller size is determined. Such measurements provide certain size-distribution data and appear to be simple and direct. However, close study of sieving has revealed serious shortcomings. Aside from the necessity for controlling such features as time of sifting and sieve load, other difficulties are inherent in the method in its application to flour. The tendency of flour to agglomerate seriously interferes with accuracy. Dusting causes errors in the small-size range. Sieves are not accurate beyond a lower limit of approximately 200-mesh. Since a large portion of the total flour weight normally lies below this limit it must be concluded that the procedure is not satisfactory for complete characterization of particle size distribution.

Of the many other methods described in the literature, liquid- and air-elutriation methods do not appear to be feasible for flour, nor do adsorption methods. High-speed centrifuging techniques are suitable for particles of very fine size, but the equipment required is both elaborate and expensive. There remain to be considered many modifications of the technique based on the rate of sedimentation in a liquid medium and measurement by microscopic observations.

Microscopic methods have been extensively studied and described by Work (1928). This procedure is, of course, fundamental in that it gives a direct measure of particle size. However, the preparation of truly representative samples for microscopic observation is difficult, and it therefore becomes necessary to measure a large number of particles, preferably from a replicated series of samples from the material under test. In order to facilitate this type of procedure,

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many investigators have used accessory devices such as the projection of an image of the specimen on a ruled wall screen, or the preparation of greatly enlarged prints of the microscope field.

The use of a sedimentation method requires relatively few assumptions and permits the determination of small particle size without excessive tedium. The fundamental work in the development of this type of procedure was carried out by Odén *et al.* (1916, 1920, 1925, 1926, 1927), who developed the theoretical basis of this method and worked out in detail an apparatus suitable for use with soils.

There are a number of methods available for determining the amount of material that reaches the bottom of a sedimentation column within a given time. Thus Knapp (1931, 1934) devised a very ingenious apparatus based on the determination of changes in hydrostatic pressure immediately above the bottom of the column. This procedure is valid because such pressure decreases in proportion as particles settle below the point of measurement. While the apparatus described by Knapp is elaborate, it has the advantage of providing an automatic photographic record. Odén and coworkers, Calbeck and Harner (1927), and Markley (1934) have described procedures in which the weight of material settling is determined by counterbalancing a pan in the suspension and determining increase in weight as the particles settle out. Calbeck and Harner's paper is of particular value for its discussion of the method of plotting and interpreting the data.

Gründer and Sauer (1937) have devised an optical method for measuring the rate of settling of flour. In one paper they describe apparatus in which a Pulfrich stufenphotometer is used to measure the light transmission of a sedimentation column at various time intervals, stating the precautions necessary for satisfactory results. They show, as might be supposed, that absorption of light depends upon particle size. The same authors later describe a refinement of the Pulfrich stufenphotometer method in which a photronic cell is used. In general, the procedure resembles the visual method but instead of photometer readings one obtains electrical potential measurements in millivolts. The authors found that particles of flour smaller than 40 μ in diameter settled very slowly and that below this size differences in settling rate were of small magnitude. For this reason they did not make measurements below 30 μ. In their samples the quantity of flour having a particle size greater than 40 μ constituted 80% of the total and only a small fraction of a percent was smaller than 30 μ. For this reason they felt that measurement of particles having a smaller diameter than 30 μ could be neglected. The papers by Gründer and Sauer provide a history of the development of a method and in the progress of its development several procedures

are shown to have possibilities. The instrument last named provided for automatic recording of a curve, and by using a standard sample the authors were able to show graphically how far other samples deviated from it.

Cereal investigators have been concerned with the possibility of a correlation between particle size distribution and baking quality. With this purpose in mind, several have devised apparatus aimed at convenience of operation rather than a high degree of precision. Included in this group are Kent-Jones (1939, 1941), Worzella and Cutler (1939), and more recently Chin (1940).

The purpose here is not to describe particle size distributions for particular flour types or to report on the relation of particle size to baking properties. Rather, the intention is to describe the application of sieving, microscopic, and sedimentation techniques to the determination of particle-size distribution in a graded series of flour samples, thus obtaining a direct comparison of results from the three types of measurement. The apparatus employed for sedimentation technique contains certain minor innovations which are believed to contribute materially to increased precision in this type of measurement.

Experimental

Preparation of samples: A bulk lot of farina obtained from a hard red winter wheat was sized by gradual reduction on the smooth rolls of an Allis-Chalmers experimental mill, the product being sifted after each passage through the rolls. In this manner, a graded series of samples, presumably of varying particle-size distribution, was obtained (see Table I).

TABLE I

PREPARATION OF FLOUR SAMPLES

Sample No.	Passed through silk No.	Retained on silk No.
A	8xx	10xx
B	10xx	11xx
C	11xx	13xx
D	13xx	14xx
E	14xx	16xx
F	16xx	—

Microscopic measurements: A Bausch and Lomb Type B microprojector assembly was employed. Essentially, this apparatus comprises a carbon arc light source, water-cell heat barrier, microscope, and a reflecting prism mounted above the ocular of the microscope in such fashion that the image of the specimen can be thrown on a screen.

On the screen was ruled a grid, the equivalent dimensions of which had been calibrated by the use of a stage micrometer. Mounts were prepared by suspending a few milligrams of sample in several drops of dilute collodion on a microscope slide, stirring vigorously with a fine-pointed glass rod, and allowing the collodion to dry. No cover glass was employed because it was found that rubbing the material under a cover glass tended to bring about a variable amount of wet milling. In the actual determination of particle size, the slide was moved unidirectionally, all particles passing across the grid of the wall screen being counted and their horizontal diameter measured to the nearest 5 μ. Only one diameter was measured for each particle, the manner of measurement being illustrated in Figure 1. The image of the

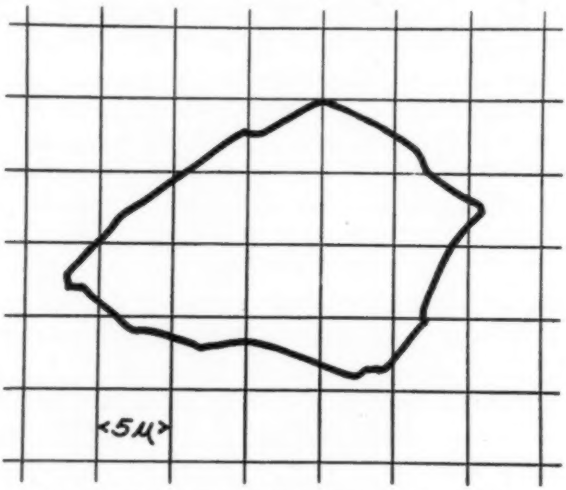

Fig. 1. Method of microscopic measurement of particle size.

particle was located on the screen so that approximately equal areas of the image lay above and below one of the horizontal grid rulings. The distance between edges of the image on this horizontal line was taken as the diameter. This procedure is justified on the assumption that the particles are oriented at random on the slide. Preliminary measurements of both horizontal and vertical diameters gave the same result, indicating the validity of this assumption.

When the full length of the mount had been traversed, the slide was moved at right angles to the first direction just sufficiently to bring uncounted particles into the field, and the process was repeated, in a direction opposite to that first employed, until a sufficient number of particles had been measured.

It was found that a substantial saving in time and effort could be effected by following a procedure suggested by Work (1928). The

particle count was arbitrarily divided into two classifications—for example, into particles larger than 20 μ and those of smaller size, the latter group containing many times more particles than the former. When a sufficient number (say 250–300) in the small sizes had been counted, the number of particles in each class for the entire distribution was recorded. A second count was then made in which all particles less than 20 μ in diameter were neglected, continuing this count until the total number was adequate to characterize the size distribution of the larger particles. The ratio of the totals for large particles in the two counts was then determined and the values for the smaller-size classes multiplied by the factor thus obtained. The data in Table II, while not showing a complete count, will serve to illustrate the form of computation employed.

TABLE II

ESTIMATION OF PARTICLE SIZE DISTRIBUTION BY MICROSCOPE COUNT—SAMPLE B, MOUNT 1

Size class	Number of particles			
	Original count	Second count	Corrected count	Total
μ				
0–5	111	—	1510	1621
6–10	119	—	1618	1737
11–15	27	—	367	394
16–20	19	—	258	277
Subtotal <20 μ	276	—	—	—
21–25	6	49	—	55
26–30	3	25	—	28
31–35	1	24	—	25
36–40	1	2	—	3
41–45		1	—	1
>46	4	103	—	107
Subtotal >20 μ	15	204	—	—

The second column of Table II shows values obtained in the first count, in which a sufficient number of particles of 0–20 μ in diameter were measured to establish this portion of the distribution. In the second count, only particles above 20 μ in diameter were considered. The values for the size classes below 20 μ were then multiplied by the factor 204/15 to give the results shown in the fourth column. The values in the third and fourth columns were then added to those in the second to give the complete distribution shown in the last column. The size characteristics of all samples were determined in duplicate in this manner, with at least two mounts used for each determination.

Sedimentation measurements: Measurements of particle size by sedimentation techniques are fundamentally based on Stokes' law, which may be expressed thus:

$$V = \frac{h}{t} = \frac{2gr^2(d_1 - d_0)}{9\eta}$$

where V is the rate of settling in cm/second, h the distance of fall in cm, t the time in seconds, g the acceleration due to gravity, r the particle radius in cm, d_1 and d_0 the density of flour and liquid respectively in g/cc, and η the viscosity of the liquid in poises. For convenience, this expression may be rearranged to give:

$$t = \frac{7.5 \times 10^6 h\eta}{gr^2(d_1 - d_0)}$$

where t is now in minutes, r in microns and the other quantities are as previously defined. For any given set of conditions this reduces to:

$$t = \frac{K}{r^2}$$

or

$$r = \frac{K'}{\sqrt{t}}$$

where K' is a constant for that set of conditions.

Thus it is possible to calculate the size of a spherical particle that will settle a given distance in a given time. If it were possible to allow a sample of flour, for example, to settle through a column of liquid, with all flour particles starting from the same level, and if the concentration of particles that had settled a given distance could be determined at various time intervals, one could then calculate directly from such data the size distribution of the flour sample. For a variety of reasons, this procedure is impractical. However, Odén (1916) has shown that such a size distribution may be calculated from data on the rate of settling of a sample homogeneously dispersed throughout the entire settling column.

Briefly, the procedure is this: Plot the weight of particles settled out as ordinate and time as abscissa. Then the intercept on the ordinate of the tangent to the curve at any time t_1 gives the weight of particles of radius equal to, or larger than, that of a particle which will just settle a distance equal to the height of sedimentation column in time t_1. By determining a number of such points of interception, the size distribution of the sample may be adequately characterized.

The actual measurements of particle-size distribution by the sedimentation technique were carried out in the apparatus shown

schematically in Figure 2. This device, which is essentially the same as that originally suggested by Odén (1916), comprises a sedimentation column in which is suspended a pan attached to the left arm of the analytical balance. The entire assembly, exclusive of the balance, is

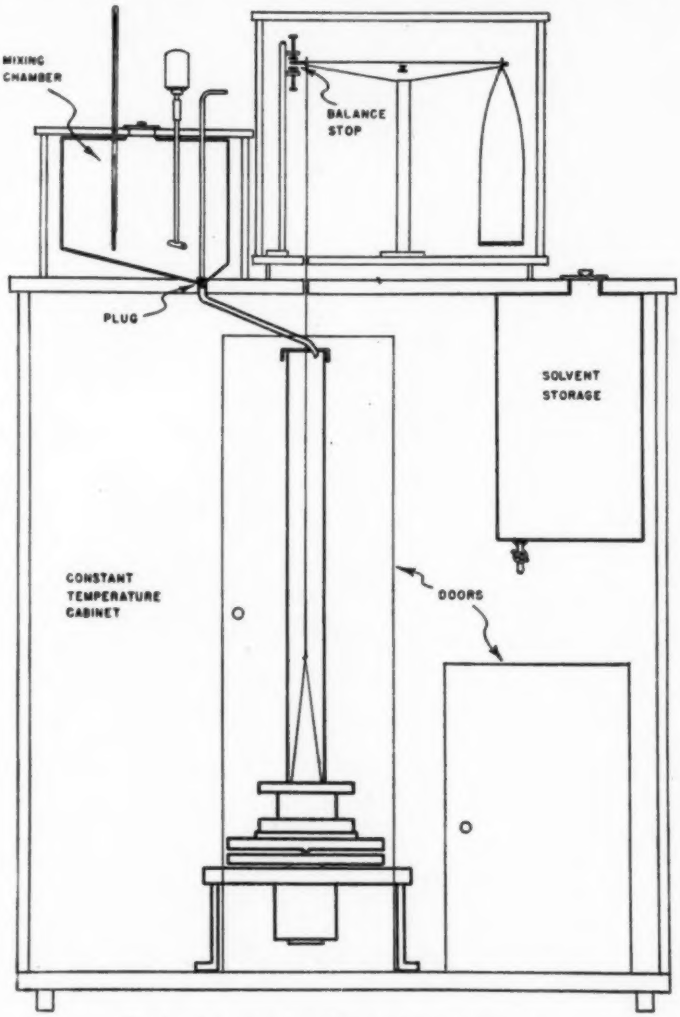

Fig. 2. Apparatus for sedimentation measurements.

contained in a cabinet provided with suitable means for circulation of temperature-controlled air. Above and to one side of the sedimentation column and connected with it by half-inch copper tubing is a small tank provided with an outlet plug, a motor-driven stirrer, and a thermometer. The base of the sedimentation column, shown in detail

in Figure 3, was designed to permit mechanical raising and lowering of the entire column for convenience in removal and also made provision for accurate centering of the pan on which settling particles accumulated.

Relatively close control of temperature (±0.1°C) is necessary to prevent variation in density and viscosity of the liquid employed.

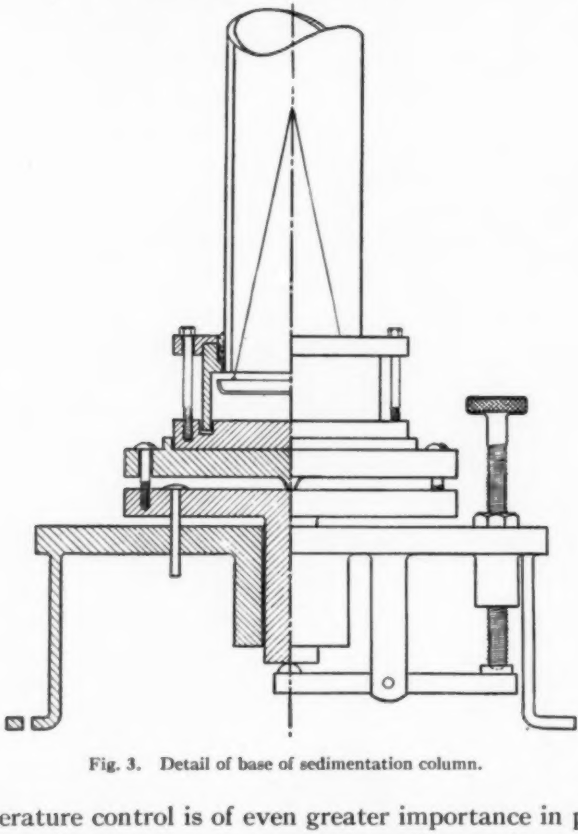

Fig. 3. Detail of base of sedimentation column.

Such temperature control is of even greater importance in preventing the formation of convection currents in the sedimentation column.

Early experiments with a pan smaller in cross-sectional area than that of the sedimentation column were found to give unreliable results, in that the total weight collected on the pan did not agree with the calculated value. Since the density of the flour sample, the density of the liquid employed, and the original sample weight are known, it is possible to calculate the total weight of flour that should settle out on a pan of known area. With pans small enough to fit within the main portion of the sedimentation column, only 60%–80% of the calculated flour weight was accumulated even after long periods of standing.

This discrepancy was not due to incomplete settling but to passage of material through the annular space between the glass tube and the edge of the pan. All attempts to modify the type of suspension employed and to alter the size of pan were unsuccessful until it was found that employing a pan larger in area than the cross section of the sedimentation column and suspending it immediately below the bottom of the column, gave total flour weights of 98%–101% of the calculated values. The reason for this behavior is not known but apparatus of this design has given entirely satisfactory results with many samples.

In carrying out the determination a suitable weight of flour, usually 10 g, was suspended in 2600 ml of liquid in the small tank. The mixture was agitated for several minutes to effect complete dispersal of the flour, the plug was then opened with the stirrer still running, and the dispersion allowed to drain into the sedimentation column. With the 75-mm-diameter glass tube which we employed, this quantity of suspension gave a 60-cm depth of liquid above the balance pan. Zero time was recorded as the time when all the liquid, with the exception of the final slow drainings, had flowed into the sedimentation column. The pan had previously been balanced while suspended in the liquid alone. A known weight (0.1 or 0.2 g) was added to the right-hand balance pan and the time recorded when the pointer swung past the balance point. The beam arrest was raised, another weight increment added to the right-hand pan, and the process repeated. The increments of weight added were varied so that a reading was obtained every four to five minutes until sedimentation was virtually complete, and a final weight reading taken after two to four hours.

Several precautions must be observed. The rate of settling must not be so great that Stokes' law is no longer valid. For ordinary flours and dispersion media, the maximum rate is well within this limiting value but some difficulty may be encountered if attempts are made to measure the size distribution of very coarse materials by this technique. The sample must be homogeneously dispersed throughout the liquid at the time that settling starts and the concentration of sample must be low enough so that the particles fall freely without colliding with each other. Obviously, the sample must be completely dispersed so that no aggregates of particles remain in the suspension, since such aggregates will settle as individual particles of that size.

In the calculation of the particle-size distribution, the accumulated weight was plotted against time on a large graph as illustrated in Figure 4. The times necessary for a series of particles of radii r_1, r_2, r_3, etc., to settle the full length of the column, were calculated from Stokes' law, as previously described, after determination of the other factors entering into this equation and evaluation of the constant K. The

times thus calculated are shown as crosses on the accumulation curve. The tangent to the accumulation curve was found graphically for each of these times and the intercept of the tangents on the ordinate determined.

It is possible to estimate the tangents to the accumulation curve arithmetically from the difference between accumulated weights corresponding to times t_1 and t_2, but this procedure is not satisfactory under conditions in which the slope of the curve is changing rapidly. The results obtained by graphical and arithmetic determination of tangents

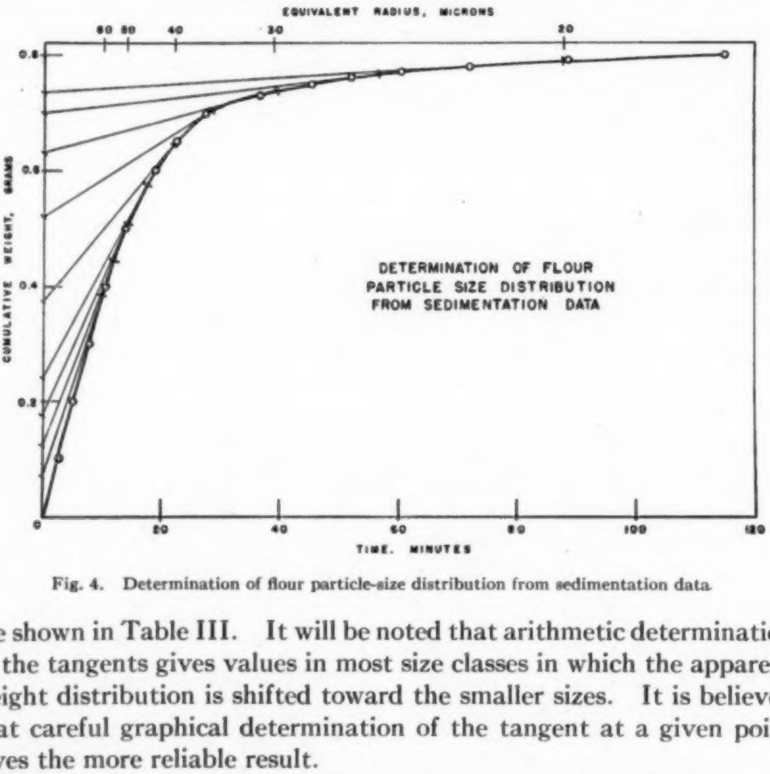

Fig. 4. Determination of flour particle-size distribution from sedimentation data

are shown in Table III. It will be noted that arithmetic determination of the tangents gives values in most size classes in which the apparent weight distribution is shifted toward the smaller sizes. It is believed that careful graphical determination of the tangent at a given point gives the more reliable result.

If an equation could be found that would fit the accumulation curve data satisfactorily, it would obviously be possible to differentiate this equation and thus obtain a satisfactory mathematical expression for the tangent and point of intercept for any given time. It has been our experience, however, that the shape of the accumulation curve changes sufficiently from sample to sample so that no general equation will fit such data with satisfactory precision, and the labor involved in fitting a special, rather complicated equation to each particular set of data is not justified by the resulting increase in precision.

TABLE III

COMPARISON OF RESULTS OBTAINED BY GRAPHICAL AND ARITHMETIC CONVERSION OF SEDIMENTATION DATA—SAMPLE F

Radius	Graphical		Arithmetic	
	Intercept	$I_n - I_{n+1}$	Intercept	$I_n - I_{n+1}$
μ	g	%	g	%
0	3.27		3.27	
		28.1		17.7
15	2.35		2.69	
		17.1		17.7
20	1.79		2.11	
		14.0		15.0
25	1.33		1.62	
		12.1		13.7
30	0.94		1.17	
		11.3		11.0
35	0.56		0.81	
		6.9		13.4
40	0.34		0.37	
		2.6		1.8
45	0.26		0.31	
		2.0		2.4
50	0.19		0.23	
		0.6		2.7
55	0.17		0.14	
		94.7		95.4

The calculation of settling times noted above requires the determination of density of both the flour and the liquid employed for the dispersion. We have found it convenient to use a pycnometer for both measurements. The density of the liquid is determined in the usual manner. The pycnometer is then cleaned and dried, a small sample of flour introduced, its weight determined by difference, and the vessel carefully filled with the liquid to be employed for the dispersion. After the vessel and its contents come to temperature equilibrium, the volume is carefully adjusted and the total weight determined. From this value is calculated, by difference, the weight of liquid required to fill the pycnometer, and from this value the volume of liquid. The difference between total volume and liquid volume is obviously that of the flour, from which value the flour density may be calculated.

The selection of a liquid to be employed for this type of determination is governed by the density and viscosity of the liquid in addition to certain special requirements. The liquid must not react with the flour in any way. It should not penetrate the flour particles, for such penetration would change the density of the flour, and it should not cause swelling of the flour particles. We have found that various

petroleum fractions may be satisfactorily employed. It is desirable to use a liquid of as low a density as is consistent with reasonable accuracy in the determination of the accumulation curve, since the greater the difference in density between flour and liquid, the smaller will be the error introduced by errors in measurements of density or by change in density with time, or with temperature fluctuation. With petroleum fractions such as gasoline, it has been observed that there is a gradual penetration of liquid into the flour particles with time. Such penetration is sufficient to change flour density by values up to 0.005 g/cc, but this variation is not of sufficient magnitude to cause significant error if the density of the liquid is less than unity.

A comparison of particle-size values given by microscopic and sedimentation measurements with those deduced from data on sieve openings is shown in Table IV. It is evident that the actual particle size

TABLE IV

COMPARISON OF SIZE RANGES FROM SIEVE OPENINGS, MICROSCOPIC AND SEDIMENTATION DATA

Sample	Sieve openings	Central 80% by weight: Microscope range	Central 80% by weight: Sedimentation range
	μ	μ	μ
A	140–193	48–116	41–83
B	125–140	56–116	34–76
C	99–125	47– 96	31–74
E	76– 94	37– 80	20–57
F	— 76	14– 82	11–45

values in all cases are much smaller than would be expected from the sieve openings. Apparently in dry sifting the particles tend to agglomerate, and the sifting behavior is then governed by the size of such aggregates. Accordingly, ultimate particle-size values of flours cannot be determined by sifting.

Typical results obtained by microscopic and sedimentation procedures on the graded series of flour samples previously described are shown in Figure 5. It is apparent that either type of measurement can be used satisfactorily to differentiate between flours, although the absolute results obtained are not the same. In all cases, microscopic measurements gave significantly larger mean particle diameters.

Consideration of the shape of flour particles affords a reasonable explanation for the difference in results. In all except the smallest particles, which are apparently free starch granules, the shape is highly irregular, the particles usually being appreciably larger in one or two dimensions than in the third. As an exaggerated case one may consider the estimation of the size of a platelike particle. In micro-

scopic measurements, such a particle would tend to settle in the mount with the two larger dimensions parallel to the surface of the microscope slide, and it is only these dimensions which are measured. In the determination by sedimentation, however, it is to be expected

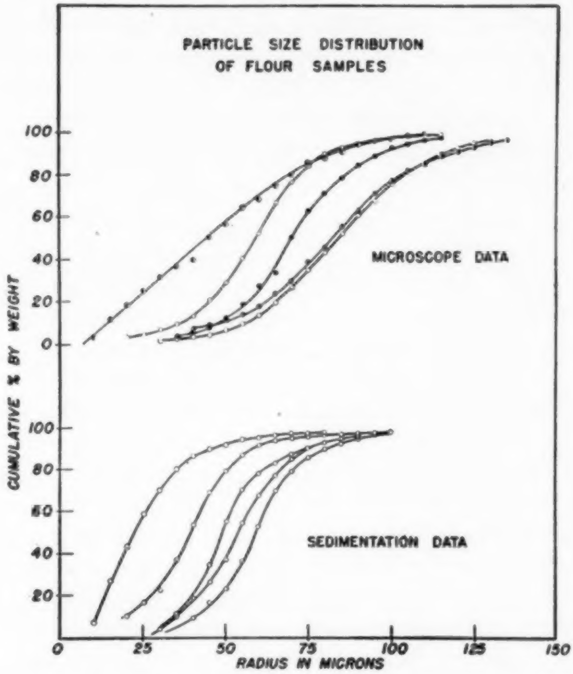

Fig. 5. Particle-size distribution of flour samples.

that the particle would settle with these dimensions parallel to the line of fall. In other words, the settling behavior of this particle would be governed largely by its smaller dimensions. Thus, in the aggregate, microscopic measurement of flour particles tends to give values that are too large and sedimentation measurements values too small. It seems that the best estimate would be the average between data obtained with the two types of measurement. For comparative studies of flours it appears that either could be employed, but the labor involved in microscopic measurements is many times greater than that necessary by the sedimentation technique.

Summary

Determinations of particle-size distribution have been made by both microscopic and sedimentation procedures on a series of flours produced from one bulk lot of farina and sized by sifting.

Values obtained by both microscopic and sedimentation techniques were much lower than would be predicted from the size of sieve openings. Flour particles are therefore not completely deflocculated in sifting, and aggregates rather than individual particles determine behavior.

The microscopic and sedimentation techniques gave different but well correlated results, the larger values being obtained with the former. An explanation is advanced for this discrepancy. True values are believed to lie between those given by the two methods.

Either procedure may be employed, but the sedimentation technique is much less laborious.

Acknowledgment

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THE EFFECT OF STORAGE OF CORN ON THE CHEMICAL PROPERTIES OF ITS PROTEINS AND ON ITS GROWTH-PROMOTING VALUE[1]

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Since large quantities of wheat, corn, and other grains are stored over long periods in warehouses and granaries of various types under the Federal ever-normal granary program, any information on the extent and nature of deteriorative changes that occur in stored grain is of special value. Studies of the kind, rate, and extent of storage changes in grain should yield information that will aid in determining the type of bins needed and storage conditions that will result in minimum deteriorative changes. Also a knowledge of the specific kinds of changes that occur may suggest the best use for grains that have deteriorated in storage—whether for food or for other purposes. The results of tests herein reported, while not extensive enough to justify specific recommendations, nevertheless throw additional light on some aspects of grain storage.

In a previous paper (Jones and Gersdorff, 1941) it was shown that when wheat and its milling products are stored, marked changes occur in the properties of the proteins. These changes include (1) decrease in the solubility of the proteins in various dispersing agents, such as neutral salt solutions and alcohol; (2) proteolysis, or breaking down of the native proteins into entities of smaller molecular dimensions; and (3) decrease in digestibility when treated with pepsin and trypsin *in vitro*. The extent of the changes depends on temperature, type of container, and on whether the whole-wheat kernels or their milling products are stored. The present paper describes the effect of similar storage on the chemical properties of the proteins of corn and on the growth-promoting value of stored corn as determined by feeding experiments with albino rats.

The results of the studies on corn here presented show that during storage marked changes occur in the properties of corn proteins. They also indicate a decrease in growth-promoting value of corn as a result of storage. The protein chemical changes are largely of the same order, rate, and extent as those previously found for wheat and wheat flour stored under the same conditions as those here described for corn.

[1] Preliminary notes on the effect of storage on the proteins of whole and ground seeds have been previously published (Jones and Gersdorff, 1938 and 1939).

Chemical Effects

Material: The shelled Yellow Dent corn used in these studies was grown on the U. S. Department of Agriculture experimental farm at Arlington, Virginia, and was obtained through the kindness of Dr. Merl T. Jenkins of the Division of Cereal Crops and Diseases, Bureau of Plant Industry. Immediately after harvesting, the mature corn was dried on the cob at 89°F in an open room in an air current generated by electric fans. The dried, shelled corn was delivered September 30, 1938.

Analysis of the corn a few days after receipt gave the following results: moisture 10.59%, nitrogen 1.87%, and fat 4.77%. (The figures for nitrogen and fat are on a moisture-free basis.)

Preparation and storage of the material: A portion of the corn was first cracked in an electric-driven Hobart mill, then ground coarsely, and finally reduced to a fine meal (October 4, 1938).

On October 5, 1938, samples of the ground corn in air-tight Mason jars and in closely woven cotton bags were stored in dark cupboards in a room maintained at a temperature of 76° ± 3° or 4°F and at a relative humidity of about 55%. Similar samples were stored in a refrigerating room maintained at a temperature of 30°F. Each jar of the ground corn contained enough material to provide for the required analyses at the end of the various storage periods. Samples of the whole kernels were also stored in two-quart Mason jars at 76°F and 30°F.

Analyses: Analyses of the ground corn samples stored in jars were made at the end of 1, 3, 6, 12, and 24 months of storage, and of those stored in bags at the end of 6, 12, and 24 months. Portions of the samples of whole-corn kernels were ground to the approximate fineness of the corn meal and analyzed after storage periods of 12 and 24 months.

Procedure: The various analyses made on the stored samples were chosen as suitable for revealing the nature and extent of changes in the properties of the proteins that might occur during storage. Proteins readily undergo changes of three common and well known kinds when subjected to the action of chemical and physical agents. One of these is degradation through hydrolysis or proteolysis, characterized by progressive breaking down of the protein into products of smaller molecular size, namely peptides of decreasing dimensions and finally amino acids. The degradation of protein is commonly accomplished by the action of acids or alkalies, and by proteolytic enzymes. Another kind of change, *denaturation*, is commonly characterized by decrease in solubility of the proteins in reagents which readily disperse them in their native or unchanged state. There may also be *changes*

in digestibility of proteins. It is known that the digestibility of many proteins is affected by the action of heat, light, alcohol, and other agents.

To learn the extent of denaturation during storage, changes in solubility of the proteins were determined by periodically extracting portions of the samples with 3% NaCl solution, 70% alcohol, and 3% sodium salicylate solution, and determining total nitrogen in the extracts.

The extent of proteolysis was measured by determining the "true-protein value" (protein nitrogen) according to the copper hydroxide method of Stutzer (1881). By means of this method the native unchanged protein is converted into an insoluble copper derivative which can be separated from the break-down products.

Digestibility was measured by the amount of protein (as represented by nitrogen) rendered soluble by digesting the samples with pepsin and trypsin.

Determinations of moisture, total nitrogen, and free ammonia nitrogen also were made on the fresh material, and on the samples at the end of the different storage periods.

The first analyses were made on the fresh samples at the time they were placed in storage. The results of these analyses served as a basis of comparison with similar data obtained on the stored samples. Throughout the investigation special care was taken to have all the corresponding analyses made in the same way and under the same conditions in order to have the results of the different sets of analyses as comparable as possible. The samples of stored corn kernels were ground to a meal in a hand-driven mill just before analysis, care being taken to have the material reduced to the same degree of fineness for each set of analyses.

The details of the methods used in carrying out the different analyses and procedures have been described in a previous paper on the effect of storage on the proteins of wheat and wheat flour (Jones and Gersdorff, 1941).

Discussion of chemical effects: At no time during the storage periods was there any visible indication of spoilage or deterioration of the material. No evidence of mold or insect infestation was apparent in the samples used for chemical tests. Some of the ground-corn samples stored in bags at 76°F had developed a slightly sharp odor, and those stored at 30°F had acquired a little "off" odor due to absorption from other material stored in the refrigerating room.

The results of the analyses of the ground corn and of the corn kernels made at the time they were placed in storage and at the end of the storage intervals are shown in Tables I and II. These results are

TABLE I

EFFECT OF STORAGE ON THE PROTEINS OF GROUND CORN
(Results expressed in milligrams per 100 grams material)

Determinations	Values for the fresh material	Months at 30° F. 1	3	6	12	24
GROUND CORN STORED IN JARS						
Moisture	10,590	10,620	10,630	10,590	10,800	10,590
Total nitrogen	1,870	1,870	1,870	1,870	1,870	1,870
True protein nitrogen	1,530	1,478	1,380	1,359	1,327	1,280
Free ammonia nitrogen	36.8	36.5	36.1	36.8	36.5	36.8
Nitrogen soluble in 3% NaCl	462	368	357	347	331	312
Nitrogen soluble in 70% alcohol	476	350	301	287	277	263
Nitrogen soluble in 3% sodium salicylate	714	620	557	494	451	410
Nitrogen rendered soluble by peptic-tryptic digestion	1,775	1,767	1,746	1,688	1,638	1,618

Determinations	Values for the fresh material	Months at 76° F. 1	3	6	12	24
Moisture	10,590	10,620	10,630	10,620	10,860	10,630
Total nitrogen	1,870	1,870	1,870	1,870	1,870	1,880
True protein nitrogen	1,530	1,464	1,324	1,219	1,186	1,112
Free ammonia nitrogen	36.8	36.1	36.1	36.8	36.8	37.2
Nitrogen soluble in 3% NaCl	462	347	326	294	263	232
Nitrogen soluble in 70% alcohol	476	336	287	259	229	210
Nitrogen soluble in 3% sodium salicylate	714	515	462	389	306	249
Nitrogen rendered soluble by peptic-tryptic digestion	1,775	1,724	1,702	1,624	1,537	1,444

Determinations	Values for the fresh material	Months at 30° F. 6	12	24	Months at 76° F. 6	12	24
GROUND CORN STORED IN BAGS							
Moisture	10,590	13,100	12,850	14,120	9,970	10,460	9,930
Total nitrogen	1,870	1,880	1,880	1,890	1,870	1,870	1,870
True protein nitrogen	1,530	1,313	1,270	1,243	1,135	1,098	1,034
Free ammonia nitrogen	36.8	36.8	37.0	37.0	36.5	36.8	37.0
Nitrogen soluble in 3% NaCl	462	326	308	301	252	229	188
Nitrogen soluble in 70% alcohol	476	265	258	242	231	210	189
Nitrogen soluble in 3% sodium salicylate	714	420	341	289	326	286	235
Nitrogen rendered soluble by peptic-tryptic digestion	1,775	1,638	1,595	1,533	1,458	1,386	1,257

expressed in milligrams per 100 g of the fresh and stored material, and are calculated to a moisture-free basis. For convenience in comparing and visualizing the changes at different periods and under different conditions of storage some of the results shown in Tables I and II are summarized in Table III, in which the figures represent decreases calculated as percentages of the values found for the fresh material.

TABLE II

EFFECT OF STORAGE ON THE PROTEINS OF CORN KERNELS

(Results expressed in milligrams per 100 grams material)

Determinations	Values for the fresh material	Stored in jars			
		Months at 30°F		Months at 76°F	
		12	24	12	24
Moisture	11,360	11,360	11,360	12,030	11,450
Total nitrogen	1,870	1,870	1,880	1,870	1,880
True protein nitrogen	1,530	1,416	1,373	1,341	1,275
Free ammonia nitrogen	36.8	36.7	37.0	36.6	36.8
Nitrogen soluble in 3% NaCl	462	383	377	362	343
Nitrogen soluble in 70% alcohol	476	347	335	321	288
Nitrogen soluble in 3% sodium salicylate	714	537	515	489	435
Nitrogen rendered soluble by peptic-tryptic digestion	1,775	1,751	1,734	1,742	1,710

The values for total nitrogen and ammonia nitrogen remained practically constant in all of the samples. Moisture content did not change, except in the case of the material stored in bags. All of the other determinations given in Tables I and II show progressive changes when compared with similar determinations at the ends of preceding periods of storage. The samples stored in bags which permitted access of air showed greater changes than did those stored in air-tight jars. Temperature was also an important factor. Although significant changes occurred in the samples stored at 30°F, they were less than those in the samples stored at 76°F. The effect of storage was much greater on the ground corn than on the unground kernels.

An inspection of the tables will show that the most striking change was the marked decrease in solubility of the proteins.

Another extensive change that took place during storage was a progressive breaking down of the native protein into smaller fragments. This change was measured by determining the so-called true-protein content by the copper hydroxide method. As in the case of the solubility changes, the decreases in true-protein values were greater in the samples stored in bags than in those stored in jars, and greater

TABLE III

EFFECT OF STORAGE ON THE PROTEINS OF GROUND CORN AND CORN KERNELS
(Results expressed as percentage decreases)

Determinations	Months at 30°F				Months at 76°F			
	1	3	12	24	1	3	12	24
GROUND CORN STORED IN JARS								
	%	%	%	%	%	%	%	%
True protein nitrogen	3.4	9.8	13.2	16.3	4.3	13.5	22.4	27.3
Nitrogen soluble in 3% NaCl	20.1	22.7	28.3	32.5	24.9	29.4	43.1	49.8
Nitrogen soluble in 70% alcohol	26.5	36.7	41.8	44.7	29.4	39.7	51.9	56.0
Nitrogen rendered soluble by peptic-tryptic digestion	0.4	1.6	7.7	8.8	2.8	4.1	13.4	18.6
GROUND CORN STORED IN BAGS								
		6	12	24		6	12	24
True protein nitrogen	—	14.1	17.0	18.7	—	25.7	28.2	32.4
Nitrogen soluble in 3% NaCl	—	29.4	33.3	34.8	—	45.4	50.4	59.3
Nitrogen soluble in 70% alcohol	—	44.3	45.8	49.2	—	51.4	55.9	60.3
Nitrogen rendered soluble by peptic-tryptic digestion	—	7.7	10.1	13.6	—	17.8	21.9	29.2
CORN KERNELS STORED IN JARS								
			12	24			12	24
True protein nitrogen	—	—	7.4	10.3	—	—	12.3	16.6
Nitrogen soluble in 3% NaCl	—	—	17.1	18.4	—	—	21.6	25.7
Nitrogen soluble in 70% alcohol	—	—	27.1	29.6	—	—	32.5	39.5
Nitrogen rendered soluble by peptic-tryptic digestion	—	—	1.4	2.3	—	—	1.8	3.6

when stored at 76°F than at 30°F. Changes in the true-protein values of the stored corn kernels, like the solubility changes, were less than in the stored ground corn, amounting to only a little over half as much.

Decrease in solubility of the proteins in dispersing agents (denaturation) during storage, and increase in soluble nitrogen due to proteolysis as measured by the Stutzer copper hydroxide method, represent two types of changes producing opposite effects. The substantial over-all decrease in protein solubility probably is, therefore, the resultant of two types of protein alteration, one tending toward an increase in soluble nitrogen and the other toward a decrease, with the latter predominating. Accordingly, it might be concluded that the extent of protein denaturation was actually greater than is indicated by the solubility data.

Decreases in digestibility were observed in all of the stored samples, the extent varying according to the type of container and storage temperature. The changes in the corn kernels, however, were small.

The rapid rate at which the changes occurred during the early periods of storage deserves emphasis. Decreases in true protein and in solubility were much more rapid during the early storage periods than later. Ground corn stored in bags changed even more rapidly than the samples stored in jars. The changes in the corn kernels also apparently developed more rapidly during the first few months of storage than later. Analyses were not made on the stored kernel samples until after 12 months' storage.

The effects of storage on the proteins of corn were very similar in type and extent to those previously described for wheat and wheat flour stored under practically identical conditions. Decreases in solubility and digestibility and the extent of protein breakdown were about of the same order in wheat and corn. The changes were much greater in the flour than in the wheat kernels, and greater in the samples stored in bags than in those stored in jars. The effect of storage was also much more marked during the early storage intervals than later. The changes that occurred during the first month of storage were in some instances as much as three-fourths of those found at the end of 24 months.

The probable causes of the changes occurring in the proteins of wheat during storage were discussed in the publication already referred to, in which the changes were ascribed to the effects of enzymic action and oxidation. Since the storage effects on the proteins of corn are so similar to those on the wheat proteins, it seems highly probable that they are attributable to the same causes.

Nutritional Effects

Concurrently with the chemical studies, feeding experiments with albino rats were conducted in order to see what effect, if any, storage might have on the nutritive value of the proteins of corn. The materials used and the conditions of storage were the same as those already described under "Chemical Effects."

About 80 pounds of corn was ground to a fine meal in an electrically driven mill, care being taken to avoid heating. One-half of the meal was placed in two-quart Mason jars (made air-tight with rubber rings) and stored in a dark cupboard in an air-conditioned room maintained at 76°F and 55% relative humidity. The remaining half of the meal was placed in cotton bags and stored under similar conditions. Whole corn kernels, in two covered galvanized iron cans sealed with adhesive

tape, were also stored at 76°F. The corn, freshly ground, contained 10.42% protein (N × 6.25) uncorrected for moisture.

With only one exception there was no indication of mold, insect infestation, or of any apparent deterioration in the samples during storage as indicated by "off" odor or color. One sample of corn kernels stored in a can became infested with weevils. The results obtained with this sample (Lot 201, Table IV) are, therefore, invalid.

TABLE IV

EFFECT OF STORAGE ON THE GROWTH-PROMOTING VALUE OF GROUND CORN AND OF WHOLE CORN KERNELS

Lot No.	Storage conditions	Storage periods	Initial weight	Final weight	Food consumption	Gain in 42 days	Gain per g protein eaten
		mos	*g*	*g*	*g*	*g*	*g*
93	Fresh unstored corn	—	56	140	551	84	1.52
99	Fresh unstored corn	—	55	138	542	83	1.53
124	Meal in jars at 76°	6	57	120	415	63	1.52
125	Meal in bags at 76°	6	58	126	446	68	1.52
149	Meal in jars at 76°	12	57	113	414	56	1.35
150	Meal in bags at 76°	12	58	111	393	53	1.35
202	Meal in jars at 76°	24	57	114	419	57	1.36
201	Meal in bags at 76°	24	56	110	409	54	1.32
151	Kernels in can at 76°[1]	12	57	103	394	46	1.17
203	Kernels in can at 76°	24	56	112	448	56	1.25

[1] This sample contained weevils.

Feeding tests on the freshly ground corn were started within a day or two after grinding. Subsequent tests were started on the ground corn samples at the end of storage intervals of 6, 12, and 24 months. The stored whole kernels were fed at the end of storage intervals of 12 and 24 months.

Young albino rats (8 to each lot), weighing 55 to 60 g each and equally distributed as to sex and litter mates, were fed the rations over 42-day periods. The animals were weighed twice weekly. On each weighing day the unconsumed feed was discarded, the cups were replenished with fresh diet, and the weight gains and food intake of the animals were recorded.

The diet consisted of 96 parts corn, 3 parts salt mixture (Osborne and Mendel), and 1 part cod-liver oil. It contained 10% of protein, all of which was upplied by the corn. A solution of vitamins, freshly prepared each week, was given orally to each rat by means of a syringe. Two-tenths ml of this solution given daily contained 10 μg thiamin, 10 μg riboflavin, 0.05 mg nicotinic acid, 25 mg "Ryzamin B,"[2] and 15 mg sucrose.

[2] An extract of rice polishings obtained from Burroughs, Wellcome and Co.

Discussion of nutritional effects: The results of the feeding experiments are shown in Table IV. The figures given for each lot are the averages of fairly closely agreeing values obtained with 8 rats. The data show significant decreases in weight gains as the length of time during which corn or meal was stored increased up to 12 months.

The average gain in weight of rats in Lots 124 and 125 fed the meal stored 6 months in jars and in bags amounted to only 63 g and 68 g, respectively, as compared with 84 g for rats in Lot 93 fed the fresh unstored material. These values represent decreases of 25% and 19% in average weight gains of rats fed stored material as compared with those fed fresh material. The gain in weight per gram of protein eaten, however, was the same (1.52 g), but the consumption of the stored meal was definitely less. The meal may have become less palatable as a result of storage. The decrease in weight gains could have been caused by the lower food consumption rather than by an actual decrease in nutritive value. Either case would represent a deterioration from the standpoint of feeding value.

With meal stored similarly for 12 months, the values for average weight increases for rats in Lots 149 and 150 were 33% and 37% less than that for rats fed the fresh unstored meal (Lot 93). Although consumption of the stored meal was less than that of fresh meal, nutritive value was also less, since the gain per gram of protein eaten was less by about 11%. The average gain in weight of rats in Lots 202 and 201 fed meal that had been stored for 24 months remained essentially the same as for those fed meal stored for 12 months (Lots 149 and 150). The variations shown are considered within the limit of experimental error. With meal that had been stored longer than 6 months the decreases in average weight gain were somewhat greater for the samples stored in bags than for those stored in jars.

The effect of storage on the growth-promoting value of the proteins of whole corn kernels over a storage period of 24 months (Lot 203) was the same as that found for the ground corn stored for the same period. Unfortunately, weevils got into the sample stored for 12 months (Lot 151), which probably accounted for the low average growth increment of 46 g.

A comparison of the effect of storage on the chemical properties of the proteins with that upon their growth-promoting value is shown graphically in Figure 1. An interesting similarity in the profiles lie in the fact that the greater part of the changes occurred during the early periods of storage. About 75% of the decrease in average weight gain at the end of 24 months had already occurred by the end of the first 6-month storage period in the meal stored in jars at 76°; the corresponding ratio for the meal stored in bags was about 50%.

Similarly, the corresponding ratios for decreases in solubility in NaCl, true protein, and digestibility for the meal stored in bags were about 75%, 43%, and 60%, respectively.

It should be emphasized that the corn used in these studies, as already noted, was carefully dried, shelled, and placed in storage very soon after it had been harvested. If the same corn after harvesting had been shocked and allowed to remain in the field over a period of time, as is the common farm practice, a considerable part of the changes observed at the end of the first month's storage period would probably have already taken place.

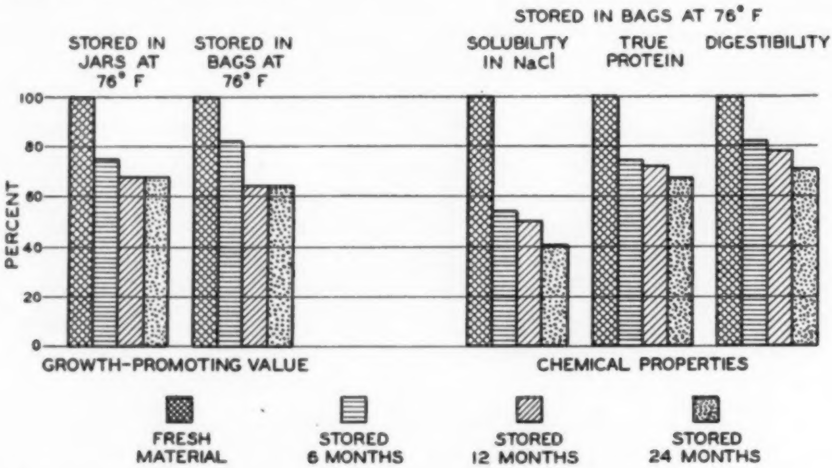

Fig. 1. Decrease in growth-promoting value and changes in chemical properties of the proteins of ground corn as a result of storage for various periods.

The cause of the decrease in growth-promoting value of the corn proteins on storage is not clear. The nature of the chemical changes observed throws little, if any, light on this question. Denatured proteins as a rule are more digestible than native proteins, and proteolysis is one of the first steps involved in protein digestion in the alimentary tract. The fact that the food consumption by rats fed with fresh corn was much higher than that by rats fed with stored material suggests that some change occurred that decreased palatability. It is to be noted that during the storage periods there was a marked progressive increase in free fatty acids as determined in the oil extracted from samples of the stored corn. It does not seem, however, that the decrease in weight gains can be wholly accounted for by impaired palatability. As shown in Table IV there was also a decrease in gain in weight per gram of protein eaten after the first six months of storage. The ease with which proteins are known to be

altered by relatively mild influences suggests that the same factors that brought about the observed chemical changes in the properties of the proteins also could have impaired the integrity of one or more of the nutritionally essential amino acids, or rendered them unavailable for assimilation.

Summary

The effects of storage of ground corn and of whole shelled corn upon the properties of the proteins and upon nutritive value were determined at various intervals over a storage period of two years. The results show that three different types of alterations in the proteins occur: (1) a decrease in the solubility of the proteins, (2) a partial breakdown of the proteins, indicated by a decrease in true protein content, and (3) a decrease in digestibility.

The extent of the alterations is influenced by temperature, type of container, duration of storage, and the nature of the material stored. Samples stored at 76°F were affected more than those stored at 30°F, and those in bags more than those in sealed glass jars. Changes in the ground corn were greater than those in the whole shelled corn. The total nitrogen and free ammonia remained unchanged. The extent of denaturation of the proteins was measured by determination of solubility in 3% NaCl solution, 70% alcohol, and 3% sodium salicylate. At the end of two years' storage in a bag at 76°F the solubility of the ground-corn protein in NaCl solution and in alcohol was approximately 60% less than that of the fresh material. The true-protein value and digestibility had decreased 32% and 29%, respectively. The rates of the decreases were much more rapid during the early storage intervals than later.

Significant decreases in feeding value were also found. During a 42-day feeding period the average gain in weight of albino rats fed with ground corn which had been stored for 6 months in jars at 76°F was 63 g in comparison with 84 g for rats fed with the fresh material, and 56 g for rats fed with material stored for 12 months. The value remained the same at the end of the second year's storage. In every instance less of the stored material than of the fresh material was consumed by the same number of rats in the same time. For material stored 6 months, the gain in weight per gram consumed was the same as for fresh material. For material stored 12 and 24 months, the gain in weight per gram consumed was less.

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CHEMICAL FACTORS AFFECTING THE BAKING QUALITY OF DRY MILK SOLIDS. II. THE EFFECT OF MILK ON GLUTEN FRACTIONATION [1]

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Almost all dry milk solids now being produced are of good baking quality (Ashworth, Golding, Farrah, and Miller, 1942). However, an occasional sample is found that produces poor loaf volume. An important step toward the correction of this difficulty would be that of finding the nature of the effect of the milk on the dough.

There is no doubt that the effect of proteolytic enzymes is of major importance in determining the quality of bread (Balls and Hale, 1936, 1936a; Jørgensen, 1936). However, the amount of proteolysis ordinarily encountered in baking is so small that no suitable method of measurement, short of the baking itself, is known. Recent reports on the methods for determining the amount of proteolytic enzymes in flour are those of Hildebrand (1940) and Landis (1941).

The method of gluten dispersion in sodium salicylate solution developed by Rose and Cook (1935), McCalla and Rose (1935), Harris (1937, 1938, 1939), and Harris and Johnson (1939, 1940, 1941) seems to be one of the most sensitive found for determining the effect of proteolytic activity. We have used two modifications of the method to furnish evidence of the nature of the action of milk on flour gluten and the effect of milk on the action of papain.

Methods and Materials

The methods of Harris and Johnson (1940) with slight modifications were followed in the first part of this investigation. The dough contained 100 g flour, 3 g yeast, 1.5 g sodium chloride, 3.5 g sucrose, and water as required. The milk doughs contained 6.0 g of dry milk solids. Four mg of Merck's papain was added to one-half of the

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doughs just before mixing. They were mixed one minute and fermented one hour.

Following fermentation the doughs were washed with 0.1% phosphate buffer (Dill and Alsberg, 1924), and the gluten dispersed in 10% sodium salicylate solution by gentle agitation on a mechanical shaker for 22 hours. The undispersed gluten and starch were removed by centrifuging. The nitrogen concentration of the dispersion was determined and adjusted to 4.0 mg per milliliter of dispersion. A fraction of the gluten was precipitated by the addition of 1.000 g of magnesium sulfate to 25 ml of dispersion. After standing one hour the precipitate was centrifuged and washed with 10% sodium salicylate containing 1.000 g of magnesium sulfate per 25 ml. The nitrogen content of the precipitate was then determined.

The effect of dry milk solids, in both the presence and the absence of 4 mg % of papain, on the amount of protein nitrogen precipitated by magnesium sulfate solution was studied in the first part of the investigation.

For the second part of the investigation 2.000 g of flour was weighed into 50-ml centrifuge tubes. Five ml of water was added and after thorough stirring the tubes were allowed to digest for 4 hours at 37°C. The digesting mixtures were stirred at 30-minute intervals. Milk, papain, and yeast, when added, were first suspended in the water. All additions made were calculated as a percentage of the flour used. The amounts of papain added were 5 and 50 mg per 100 g of flour. However, since 1.7 times as much water was used in the fractionation of the gluten as could be used in baking, this made the effective concentration of papain, as compared to that in the baking formula, 3 and 30 mg respectively. When yeast was used its amount was 3% added with 5% sucrose and 1.5% salt.

At the end of the digestion period 30 ml of buffer solution (Dill and Alsberg, 1924) was added and the mixture thoroughly agitated. The gluten was allowed to settle for 15 minutes. Then 25 ml of the supernatant liquid was carefully poured off into a graduated cylinder. Four more washings were made with 25-ml portions of the buffer and one with distilled water, with 15 minutes between washings allowed for the gluten to settle. Before the final wash water was decanted, the mixture was centrifuged to pack the gluten in the bottom of the tube; then all the wash water was poured off. A nitrogen determination was made on the combined washings. Also the nitrogen content of all the additions made (such as milk, yeast, and enzymes) was determined so that their nitrogen content could be subtracted from the nitrogen content of the washings. The difference was assumed

to be the fraction of the flour nitrogen soluble in the phosphate buffer at pH 6.8.

For the second fraction 20 ml of 10% sodium salicylate was added to the gluten remaining in the centrifuge tube. The gluten was thoroughly mixed in the dispersing agent with a glass rod and agitated at 15-minute intervals for one hour. After standing overnight the mixture was shaken once and after 30 minutes more was centrifuged 20 minutes at 2600 rpm. The dispersed phase was poured into a Kjeldahl flask for nitrogen determination.

Finally the remaining gluten in the centrifuge tube was quantitatively transferred to another Kjeldahl flask and its nitrogen content determined. This constituted the insoluble residue fraction.

To check the method the sum of the three nitrogen fractions was compared with the nitrogen content of the flour. Agreement was almost invariably within 1% of the total nitrogen content of the flour. Results showing any greater discrepancy were discarded.

Three flours were used in this investigation, flours A, B, and C, containing 14.9%, 13.7%, and 10.8% protein respectively (13.5% moisture basis). Flour A was used in the first part and flours B and C in the second part. The fresh skim milk was pasteurized skim milk secured from the college dairy. The boiled skim milk was this same milk heated in a boiling water bath to 96°C for five minutes. The dry milk solids were commercially produced by the spray process from skim milk which had been preheated before drying.

Discussion of Results

The data obtained in the first part of this investigation are summarized in Table I. Each value for the "basic formula" is the average of four replicate determinations, and each value for the milk

TABLE I

THE EFFECT OF DOUGH CONSTITUENTS ON THE PERCENTAGE OF NITROGEN PRECIPITATED BY MAGNESIUM SULFATE FROM A DISPERSION OF THE GLUTEN IN 10% SODIUM SALICYLATE SOLUTION

Description of dough	Nitrogen precipitated by magnesium sulfate with: No papain	4 mg % papain	Differences
	%	%	
Basic formula	52.1±0.23	46.1±0.36	6.0*
Basic formula +6% dry milk solids of good baking quality	45.8±0.24	38.6±0.12	7.2*
Basic formula +6% dry milk solids of poor baking quality	46.6±0.39	38.9±0.51	7.7*

* These values lie beyond the 1% level of significance.

formulas represents the average of six different samples of dry milk solids. The standard errors accompany the mean values in the table. These data indicate the degree of degradation of the gluten during mixing and one-hour fermentation of the doughs. Less protein nitrogen is precipitated from the sodium salicylate dispersions of gluten by a given quantity of magnesium sulfate when there has been a reduction in the size of the gluten micelle.

Papain significantly decreased the amount of protein precipitated both with and without milk in the dough formula. The quantitative data show that the addition of milk to the dough formula does not affect the action of papain on the gluten. Since the values in the table under the heading of differences are not significantly different from each other, milk did not activate the papain. It may be noted from the data that the milk and papain had an additive effect when used together. It is purely coincidental that 6% dry milk solids produced about the same amount of degradation of the gluten as 4 mg % of papain. The differences in the amounts of protein precipitated from gluten prepared from dry milk solids of good and poor baking quality are not significant.

Although the quantitative data suggest a similarity in the behaviors of dry milk solids and papain, the physical characteristics of the two glutens were very different while being washed from the other dough ingredients. The papain had a liquefying effect on the gluten, while the milk seemed to have a weakening and coagulating effect.

Table II summarizes the results of the fractionation of gluten by the second modification. The data are given as the percentage of the total nitrogen of the flour. They are arranged in the order of decreasing values for the percentage of the total nitrogen found in the insoluble residue. The concentrations of papain given in the table as 5 and 50 mg per 100 g of flour should be corrected for the amount of water used in this modification. Since the doughs used for gluten fractionation contained 1.7 times as much water as those used for baking, the effective concentrations of papain were only 3 and 30 mg %, respectively, in the doughs used for gluten fractionation. The nitrogen values for any additions made were subtracted from the nitrogen content of the wash-water fraction since they were all water-soluble. Of all the combinations of the various dough ingredients tried, only those in which yeast is included could be actually used in baking. The position of "no additions" in the table leads one to believe that each treatment above it in the table exerts a stabilizing effect on the dough since the amount of insoluble residue is greater. Yeast and the yeast + milk combinations were particularly effective in this stabilizing effect. Even a small amount of papain failed to

TABLE II

NITROGEN DISTRIBUTION OF FLOUR

Additions made (DMS = dry milk solids)	No. of samples	Percent of total nitrogen in:		
		Wash water	Sodium salicylate	Insoluble residue
		%	%	%
FLOUR B				
Yeast + 5 mg % papain + DMS	8	19.3±0.21	24.6±0.78	56.1±0.92
Yeast alone	4	19.5±0.29	24.5±0.37	55.9±0.39
Yeast + 5 mg % papain + fresh milk	4	16.9±0.29	29.5±0.52	53.6±0.62
Yeast + 5 mg % papain + boiled milk	3	20.3±0.29	26.2±2.06	53.5±1.54
5 mg. % papain + DMS	8	27.9±0.08	20.1±0.25	52.1±0.38
Yeast + DMS	7	18.6±0.31	31.7±0.66	49.8±0.14
Boiled milk alone	4	26.7±0.73	23.9±1.14	49.4±1.29
5 mg % papain alone	8	22.0±0.44	29.6±0.84	48.3±1.16
5 mg % papain + boiled milk	4	25.6±0.60	26.6±0.98	47.8±1.73
No additions	8	21.5±0.14	31.3±1.28	47.4±1.65
5 mg % papain + fresh milk	4	27.8±0.89	25.2±0.42	47.1±1.31
Fresh milk alone	4	29.8±0.65	24.6±0.63	45.6±1.44
Yeast + 5 mg % papain	8	20.1±0.12	35.0±1.14	44.9±1.07
DMS alone	18	26.2±0.23	29.3±0.58	44.6±1.00
50 mg % papain + boiled milk	4	39.6±0.40	48.1±0.19	12.1±0.33
50 mg % papain + fresh milk	4	35.6±0.85	52.5±0.40	11.9±0.69
Yeast + 50 mg % papain + fresh milk	4	54.7±0.68	35.4±0.56	9.9±0.14
Yeast + 50 mg % papain + boiled milk	4	72.4±0.31	17.8±0.23	9.9±0.06
50 mg % papain + DMS	10	45.4±0.67	45.2±0.44	9.3±0.11
50 mg % papain alone	5	46.6±0.63	45.4±0.63	8.0±0.13
Yeast + 50 mg % papain	4	75.6±0.12	16.6±0.10	7.9±0.06
FLOUR C				
Yeast + fresh milk	3	20.7±1.99	40.2±1.72	39.1±1.11
No additions	5	25.9±0.39	35.9±0.73	38.3±0.63
Yeast alone	3	28.6±1.00	37.9±0.66	33.7±1.08
Fresh milk alone	3	36.6±1.49	30.6±0.96	32.7±1.04
50 mg % papain + fresh milk	3	6.8±1.55	70.7±1.76	22.5±1.78
Yeast + 50 mg % papain + fresh milk	4	36.9±1 37	50.6±1.71	12.4±0.53
50 mg % papain alone	8	47.9±0.05	41.6±0.21	10.6±0.49
Yeast + 50 mg % papain	2	85.0	7.7	7.3

cause any breakdown of gluten unless it was in the presence of yeast without milk. Yeast must be classed as a powerful activator of papain, while milk seems to cause inactivation.

In general skim milk had a similar effect on the action of papain whether it was fresh, boiled, or dried. Boiled milk alone had no appreciable effect on the gluten, but fresh milk and dry milk solids both caused some breakdown. The breakdown caused by dry milk solids was definitely inhibited by the addition of 5 mg % of papain. No difference could be found between the effects of good and poor dry milk solids.

Papain in the higher concentration caused much breakdown of gluten, as was to be expected. Here again however the milk, whether it was fresh, boiled, or dried, had an inactivating effect on the papain.

Yeast alone had an activating effect, most of the nitrogen being washed out in the first fraction.

The flour C had a weaker gluten, since the amount of nitrogen left in the insoluble residue was smaller. The series of treatments was not complete but the inactivating effect of fresh skim milk on 50 mg % of papain is evident.

The results of a statistical analysis of the differences between means are presented in Table III. Increases in each fraction due to

TABLE III

Comparison of the Differences Between Means Caused by Addition of Milk

Basic treatment	Milk added	Differences caused by milk in		
		Wash water	Sodium salicylate	Insoluble residue
		%	%	%
No additions	DMS	+ 4.7†	− 1.8	− 2.8
No additions	Fresh milk	+ 8.3†	− 6.5*	− 1.8
No additions	Boiled milk	+ 5.2†	− 7.2*	+ 2.0
Yeast	DMS	− 0.9†	+ 7.2†	− 7.1†
5 mg % papain	DMS	+ 5.9†	− 9.5†	+ 3.8*
5 mg % papain	Fresh milk	+ 5.8†	− 4.4†	− 1.2
5 mg % papain	Boiled milk	+ 3.6†	− 3.0	− 0.5
50 mg % papain	DMS	− 1.2	− 0.2	+ 1.3†
50 mg % papain	Fresh milk	−11.0†	+ 7.1†	+ 3.9†
50 mg % papain	Boiled milk	− 7.0†	+ 2.7*	+ 4.1†
5 mg % papain + yeast	DMS	− 0.8	−10.4†	+11.2†
5 mg % papain + yeast	Fresh milk	− 3.2†	− 5.5*	+ 8.7†
5 mg % papain + yeast	Boiled milk	+ 0.2	− 8.8*	+ 8.6*
50 mg % papain + yeast	Fresh milk	−20.9†	+18.8	+ 2.0†
50 mg % papain + yeast	Boiled milk	− 3.2†	+ 1.2*	+ 2.0†

* Values lie beyond 5% level of significance.
† Values lie beyond 1% level of significance.

the addition of milk to the basic treatment listed in the first column are given plus values. In each case when milk was added to flour alone there was a significant increase in the amount of nitrogen washed out in the first fraction, even though all the nitrogen of the milk was assumed to be washed out in this fraction and first subtracted. Apparently this was a peptizing action similar to that of sodium salicylate since there was a decrease in that fraction. No significant change was observed in the insoluble residue fraction. When dry milk solids were added to yeast + flour there was a significant decrease (although small) in the wash-water fraction, a significant increase in the sodium salicylate fraction, and a significant decrease in the insoluble-residue fraction.

When the milks were added to flour + 5 mg % papain there was in each case a significant increase in the wash-water fraction and a

decrease in the sodium salicylate fraction. There was no significant change in the final fraction except when dry milk solids were added, when there was an increase. In each case when the milk was added to 50 mg % papain there was a significant increase in the insoluble residue. The same holds true when milk is added to the combination of yeast and papain. In fact the only significant decrease found in the insoluble residue when milk was superimposed upon a formula was in the case of yeast alone. These facts very definitely show that milk does not activate papain.

The effect of milk on the action of papain in baking is shown in Table IV. The loaf volumes show that milk inhibits the action of papain.

TABLE IV

EFFECT OF MILK AND PAPAIN ON LOAF VOLUME

Baking formula	Loaf volume
	cc
A Control	838± 4.5
B 5 mg % papain	576± 8.5
C 6% DMS	853±12.4
D DMS + papain	741± 2.4
MEAN DIFFERENCES	
A–B	262*
C–D	112*
D–B	165*

* These values lie beyond 1% level of significance.

The results obtained by the second modification of gluten fractionation seem to follow the baking more closely than the results obtained by the first method. The main difference in the results obtained by the two methods was the action of papain + milk on the gluten. In the first modification the breakdown of gluten by milk and papain was equal to the sum of the breakdowns caused by milk and papain alone. There appeared to be a neutralizing effect when the second modification was used. We believe that the second modification gives a better picture of what occurs during baking.

The coagulating effect of papain on milk is well known. Balls and Hoover (1937) have suggested the use of the milk-clotting time as a measure of the activity of papain. The effect of milk on the action of papain as observed by us may be due to this coagulating effect. In a blank test (with no flour) the milk was not visibly coagulated by papain in the concentration used in the gluten fractionation work. However, the presence of flour may alter the coagulative effect of papain on milk.

Summary

The results of two methods for gluten fractionation are presented. Both methods show that milk itself breaks down gluten but does not activate the breakdown by papain. The second method shows that milk has an inhibitory effect on the action of papain, which is confirmed by baking results. Milk prevents the activation of papain by yeast.

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CORRELATIONS BETWEEN CRUDE FIBER AND ASH OF WHEAT SHORTS

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(Received for publication June 9, 1942)

A search of available literature failed to disclose any technical information on the relation between the crude fiber and ash content of wheat shorts. In work extending over a six-month interval in our laboratory, 42 samples of shorts were analyzed for crude fiber and ash content at four- to five-day intervals, the results being calculated to the 15% moisture basis.

The A. A. C. C. one-filtration method[1] was used for the crude fiber test. The shorts were ground to pass a 1-mm sieve. This is essential, as was demonstrated by preliminary tests. The ash test consisted of incinerating a 1-g sample 75 minutes at 1100°F, the resulting ash being weighed back directly on the pan of a chain-type semimicro balance. The moisture determination was carried out on a 2-g sample heated in an air oven on an aluminum plate at 140°C for 20 minutes. The resultant dried material was, of course, used for the fat analysis and finally for the crude fiber determination.

Table I gives the ash content (X) and the crude fiber content (Y) of the 42 samples. The mean ($\bar{X}$) of the ash is 3.7300% and the mean ($\bar{Y}$) of the crude fiber is 5.6583%. The standard deviations (σ_x and σ_y) are 0.2235% and 0.4553%, respectively. It is to be noted that these deviations are a measure of the variation that may be expected in commercial samples, and not of variation due to the measuring technique. Had the object been to determine the variability of the ash and crude fiber tests as such, a series of analyses would have been run repeatedly on one sample. In this connection, the coefficient of variation (CV) for ash was 5.99% and for crude fiber 8.04%, indicating that the crude fiber was relatively more variable than the ash.

The coefficient of correlation by the method of Treloar[2] was found to be +.8858, a very high and significant positive correlation. Although this correlation is based upon a relatively small group of analyses, the group is sufficiently large and the correlation is significantly high to justify the conclusion that a definite significant positive correlation exists between crude fiber and ash values of shorts. This correlation suggests the possibility of predicting crude fiber values from the ash results (Table I). For this purpose the formula

[1] Methods of Analysis of Cereals and Cereal Products, 1928.
[2] Alan E. Treloar, An Outline of Biometric Analysis, 1936. The formula for standard deviation is found on page 16; that of coefficient of variation also on 16; correlation coefficient, page 47; rectilinear regression, page 56, and error of estimate, page 57.

TABLE I

PREDICTION OF CRUDE FIBER CONTENT IN SHORTS FROM ASH CONTENT

Ash X	Analyzed crude fiber Y	Predicted crude fiber $1.8X - 1.06$	Ash X	Analyzed crude fiber Y	Predicted crude fiber $1.8X - 1.06$
%	%	%	%	%	%
3.09	4.64	4.50	3.75	5.75	5.69
3.34	4.28	4.95	3.75	5.94	5.69
3.36	5.10	4.99	3.77	5.67	5.73
3.44	5.02	5.13	3.77	5.87	5.73
3.47	5.05	5.18	3.78	5.53	5.74
3.48	5.68	5.20	3.78	5.93	5.74
3.52	5.56	5.28	3.84	5.61	5.85
3.53	5.48	5.30	3.84	5.81	5.85
3.54	5.39	5.31	3.86	5.94	5.89
3.55	5.23	5.33	3.87	5.68	5.91
3.59	5.50	5.40	3.88	5.78	5.92
3.65	5.27	5.51	3.89	6.16	5.94
3.65	5.45	5.51	3.90	5.57	5.96
3.66	5.48	5.53	3.90	5.82	5.96
3.66	5.74	5.53	3.92	6.27	6.00
3.67	5.75	5.55	3.98	6.03	6.10
3.68	5.33	5.56	4.01	5.97	6.16
3.69	5.72	5.58	4.04	6.07	6.21
3.70	5.54	5.60	4.06	6.18	6.25
3.70	5.64	5.60	4.18	6.53	6.46
3.70	5.80	5.60	4.22	6.89	6.54
			Mean 3.73	5.6583	5.654

All results are calculated to the 15% moisture basis.

$Y = 1.8X - 1.06$ (where Y equals percent of crude fiber and X equals percent of ash) suggests itself from the data, the constants being determined from the formula of Treloar. From the standard error of estimate we can expect that the average predicted results will not vary more than 0.21% from the actual analyzed values. The formula $Y = 1.8X - 1.06$ should be useful in the routine control of fiber in shorts by enabling the control chemist to determine the relative crude fiber content quickly (in less than 90 minutes) when using the quick ash method previously described.

Preliminary work indicates that a constant can be determined that may be used to predict the probable crude fiber of wheat bran ground to pass a 1-mm sieve.

Conclusion

Evidence is presented which shows that for wheat shorts that are ground to pass a 1-mm sieve the coefficient of correlation between crude fiber and ash is +.8858.

A formula for the prediction of probable crude fiber from the ash values (percent of ash times 1.8 − 1.06 equals percent of crude fiber) has been derived from the data.

FURTHER OBSERVATIONS ON A CRYSTALLINE WHEAT PROTEIN [1,2]

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Enzyme Research Laboratory, Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture, Washington, D. C.

(Read at the Annual Meeting, May 1942)

In recent publications (Stuart and Harris; Balls, Hale, and Harris; and Coulson, Harris, and Axelrod, 1942) the preparation and some of the properties of a new crystalline proteinlike material from wheat flour were described. The crystals are the hydrochloride of a substance that appears to be related to the protamines, being of relatively low molecular weight, rich in arginine and (peculiarly) in cystine. Analyses have shown that it is made up largely, if not entirely, of amino acid residues.

This substance is on the border between true proteins and their degradation products. It is incompletely precipitated by sulfosalicylic acid and by trichloroacetic acid. Measurements of the rate of diffusion indicated a value of about 10,000 for the molecular weight but this method is notoriously prone to error. Analytical data have shown that the molecular weight must be at least 6,000.

The sulfur content of the substance is among the highest known for proteins. This sulfur, present as cystine, may be reversibly oxidized and reduced. In the original plant material at least part exists in the reduced form. Because of the prominence of the sulfur and because of the source of the material it is proposed to name this substance "purothionin" (*πυρος* wheat, *θειον* sulfur).

The present paper presents evidence indicating a peptide nature of linkages between the amino acids that make up most, if not all of the molecule. This evidence is based on the digestibility of the substance with certain proteolytic enzymes—namely with crystalline chymotrypsin, chymopapain, and papain, and with crude papain latex. All of these proteinases or mixtures thereof are known to attack CO–NH linkages, while evidence for their action on bonds of other types appears to be scanty. Exhaustive digestion with these proteinases has been found to cause an extensive breakdown of the crystalline material, as shown by increases in the amino nitrogen determinable by the Van Slyke apparatus. The extent of the degradation varies with the enzyme used and in some cases has accounted for half of the increase in amino nitrogen obtained on hydrolysis with acid. A further argument for the proteinlike nature of the substance may be based

[1] Enzyme Research Contribution No. 78.
[2] Part of this work was done under Bankhead-Jones, Special Research funds.

on the fact that the smooth-muscle-contracting property disappears on digestion by papain. (Histamine, it may be noted, is not inactivated by similar treatment.) The uterus-contracting property of the new substance from wheat is therefore referable to a configuration scissile by the enzyme, presumably at one or more peptide linkages. The toxic properties of the material depend on the manner and sequence in which certain components nontoxic in themselves are bound together in the molecule.

The technique by which the extent of proteolysis was determined consisted in measuring the increase of free primary amino nitrogen in a Van Slyke apparatus. In the case of the pancreas enzyme, chymotrypsin, this presented no difficulty. The digestion ran very slowly at pH 5.0 in the presence of acetate buffer, and much faster at pH 8.0 with phosphate buffer. Reduction of the substrate accelerated the rate of digestion at pH 5.0. There is evidence that final equilibrium is reached with this enzyme only after considerable time.

Digestion by the papainases is complicated by the presence of large quantities of cystine in the substrate. In the oxidized (S—S) form the substance is not digested at all. Instead the enzyme is markedly inhibited with respect to its milk-clotting power by the presence of the substrate. This inhibition may be removed by the addition of the usual reducing agents employed to activate papain. It seems probable therefore that the substance inactivates papain in much the same manner as would cystine itself. In order to obtain proteolysis with enzymes of the sulfur class it is necessary first to reduce the substrate. When this was done splitting occurred rapidly on the addition of the enzyme. Because this substrate precipitates in even weakly alkaline solutions, reduction with cyanide or sulfite was impractical. Reduction with hydrogen sulfide can be accomplished in acid solution and was therefore employed.

The results of experiments in which digestion was carried on in the presence of dissolved hydrogen sulfide were reported in a recent abstract. These results are incorrect because it was not then realized that the reaction between nitrous acid and hydrogen sulfide in the Van Slyke apparatus would produce enough nitrogen to make an appreciable difference in the extent of digestion as measured. More recent experiments, however, have shown that the amount of nitrogen so generated not only introduces a serious error but also a variable one, for the reason that it is difficult to keep the concentration of hydrogen sulfide constant during the course of a digestion lasting many hours.

Instead of applying corrections derived from blank experiments the following technique was used and gave satisfactory results.

"Purothionin" hydrochloride was dissolved in water and diluted with $0.1M$ acetate buffer (pH 5.0) to give a concentration near 10 mg per ml. The solution was then saturated with hydrogen sulfide for several hours, usually overnight. The hydrogen sulfide was then removed by a stream of hydrogen. The solution was then cooled in ice and to it was added a solution of the enzyme, made two-thirds molar with respect to potassium cyanide five minutes before being used. A portion of the chilled mixture was then immediately placed in the Van

TABLE I

INCREASE IN AMINO NITROGEN DURING DIGESTION WITH PROTEINASES [1]

Enzyme	Substrate [2]	Enzyme quantity	Amino nitrogen at hours shown [4]						Increase in amino N / Total amino N after acid hydrolysis
			0	3	20	25–30	45	80	
	mg NH_2-N	*M.C. units* [3]	*mg*						%
Chymotrypsin, cryst.									
(substrate in S–S form [5])	1.14	1.2	0.21	—	0.33	—	—	—	11
(substrate in S–S form)	2.28	1.2	0.33	0.34	—	—	0.37	—	2
(substrate in red. form)	1.21	1.2 [6]	0.29	0.32	0.39	0.40	0.42	—	11
(substrate in red. form)	2.36	1.2	0.34	—	0.55	0.59	0.61	—	11
(substrate in red. form)	1.21	1.2	0.20	0.25	0.32	0.34	—	—	12
Papain, cryst.	1.14	0.15	0.28	0.55	0.73	—	0.73	—	40
Chymopapain, cryst.	1.14	1.60	0.30	0.51	0.73	—	0.79	0.81	45
Papaya latex	1.14	0.33	0.34	0.56	0.76	0.76	—	—	37

[1] Analysis of 1 ml of digestion mixture at pH 5.0 containing $0.1M$ acetate and substrate as shown.

[2] The substrate used is expressed as mg of total amino nitrogen after acid hydrolysis.

[3] A milk-clotting unit is taken as the quantity of enzyme that, in a volume of 1 ml, clots 5 ml of an emulsion of powdered milk in one minute at 30°C. The emulsion is made by stirring 20 g of powdered milk with 85 ml of water containing 10 ml of $2M$ acetic acid + $1M$ sodium hydroxide buffer (pH 4.6).

[4] Time approximate only.

[5] At pH 8.0 in $0.1M$ phosphate buffer. Digestion by chymotrypsin was very slow at pH 5.0 with the S–S form of the substrate but quite rapid after reduction.

[6] The enzyme in this experiment was previously treated with KCN in the manner described for the activation of papain. This run is omitted from Table II.

Slyke apparatus (already prepared for use) for the determination of the initial amino nitrogen. The enzyme-substrate mixture was next placed in a thermostat at 35° and maintained there throughout the digestion. The splitting was measured by the withdrawal at intervals of further samples on which similar determinations of amino nitrogen were made. Subsidiary experiments showed that traces of hydrogen sulfide did not materially disturb the results obtained and that the presence of the cyanide (used for activating the enzyme) increased the rate, but not the extent, of digestion. The amino nitrogen produced

by autolysis in the enzyme preparations themselves was carefully determined in each case under the same experimental conditions. It was found to be too small to affect the results. The necessary corrections (amounting in the largest instance to 0.02 mg nitrogen) have been subtracted in the calculations.

The data of the experiments are shown in Table I. The increase in amino nitrogen caused by chymotrypsin was about one-third of that referable to the papainases. Papain and chymopapain apparently

TABLE II

Peptide Nitrogen[1] Set Free by Digestion

	Peptide N	Proportion
	%	*equivalents*
Set free by:		
Acid hydrolysis	100	6.0
Chymotrypsin (1) S-S form of purothionin	12	0.7
(2) SH form	13	0.8
(3) SH form	13	0.8
Papain	46	2.7
Chymopapain	51	3.1
Latex	43	2.6
Content of constituents:		
Total chlorine (6.5% of substance)	—	1.5
Peptide N in arginine (20.4% of substance)	—	0.9
Total sulfur (4.42% of substance)	—	1.1
Original amino N (1.65% of substance)	—	0.9

[1] Calculated as total N—(¾ arginine N + original amino N).

split the substrate at the same linkages, because digestion mixtures made with papain and chymopapain, respectively, and already at a standstill, showed no increase after being mixed together. Crude papain digested the substrate to the same extent as the crystalline enzymes prepared from it.

The meaning of these results is not very clear, however, until account has been taken of the fact that the original material contains 1.65% of free amino nitrogen. If the original amino nitrogen plus three-fourths of the arginine nitrogen is subtracted from the total nitrogen, 62.1% of the total remains. This residual value has been assumed to represent the nitrogen present in peptide linkages, and is hereafter referred to as "peptide nitrogen." It amounts to 10.8% of the original substance. This assumption is supported by the observation that the amino nitrogen found after hydrolysis with acid agrees very closely with that obtained by calculation from the arginine content determined by an independent method. Moreover, the original amino nitrogen is very nearly a simple submultiple (about one-sixth) of the peptide nitrogen.

Table II shows the increase in amino nitrogen due to digestion expressed as percentage of the peptide nitrogen of the substrate. The nitrogen equivalents of the chlorine, sulfur, and certain other constituents are also shown, calculated on the same basis. Changes in the peptide chain are thus more clearly seen.

From this table it appears that hydrolysis by chymotrypsin has approximately doubled the amount of amino nitrogen originally present. The newly formed amino nitrogen is moreover equivalent to the substrate's content of sulfur and also of arginine. The effect of the papainases is close to thrice that of chymotrypsin.

A simple numerical relationship appears to exist between these values. It is admittedly risky to rely too heavily on data obtained by enzyme action on a possibly inhibitory substrate, especially when only one method of determination has been used. In this case, however, the data correlate remarkably well with values for other constituents (chlorine, sulfur, etc.) determined by methods admittedly very accurate. It is apparent that the data of Table II are best correlated as submultiples of six. Other small integers give poorer agreement.

With chymotrypsin the protein behaves as though one out of every 6 peptide linkages were split; with the papainases, 3 bonds were split out of 6. Furthermore, on the same basis there appears to be one sulfur atom, one arginine residue, and one free amino group for every 6 bonds; while for every 12 there are 3 chlorine atoms.

Evidence is thus fairly satisfactory that at least half of the nitrogen linkages in the substrate are like those occurring in proteins. It is, of course, not necessary to suppose that degradation by papain has been uniformly to dipeptides. It is, however, improbable that such results would have been obtained if the proteinases attacked bonds irregularly placed with respect to the free amino group, the sulfur or the arginine. An attack at regularly recurring intervals throughout the substrate molecule seems to be clearly indicated.

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BOOK REVIEW

Volumetric Analysis, Volume I: I. M. Kolthoff and V. A. Stenger. Interscience Publishers, Inc., New York. Price $4.50.

This book was originally published in German in 1927 and an English translation was made by Prof. N. H. Furman and published in 1928. In 1930 a somewhat revised German edition appeared.

The present edition was prepared in English with some change in emphasis and with deletions and additions. It follows the general plan of its predecessors. Its scope is shown by the chapter titles: Fundamentals of Volumetric Analysis, The Principles of Neutralization and Ion Combination Reactions, Titration Curves for Neutralization and Ion Combination Reactions, The Principles of Oxidation-Reduction Reactions, Titration Curves, Indicators, Titration Error, Reaction Velocity, Catalysis and Induced Reactions, Adsorption and Coprecipitation Phenomena, Volumetric Methods of Organic Analysis, Methods for the Determination of the Equivalence-Point.

The chapter on indicators has been expanded and greater emphasis has been placed on oxidation-reduction indicators. Two sections of this chapter deal with acid-base and precipitation indicators. The authors consider indicators largely from the theoretical standpoint and present a good treatise for one interested in the development and use of volumetric analysis. A selected list of indicators having oxidation potentials between 0.24 and 1.30 (against the normal hydrogen electrode) is included, and also a table giving the transition intervals at 18° and 100°C for 16 acid-base indicators. Fifty-five pages are devoted to this chapter.

The following chapter contains a discussion of titration error, primarily the error that occurs because an indicator changes a little before or after the equivalence-point. Equations for the estimation of this error are developed.

A section on the formation of mixed crystals gives a detailed quantitative discussion of the errors involved. Mixed crystal formation is suggested as a method for precipitation of traces of ions from solution for subsequent volumetric determination.

A chapter of 49 pages presents methods of organic analysis. Only the non-electrolytes are discussed. Methods of saponification or hydrolysis of esters, the formation of addition and condensation products, substitution reactions and methods of oxidation and reduction are treated. The chapter suggests that further fundamental studies are needed in this field. No attempt has been made to include special procedures.

In the chapter on the determination of the equivalence-point, the authors have introduced a discussion of the principles of amperometric (polarimetric) titration with polarized electrodes.

The appendix includes among other things a table of ionization constants and solubility products.

The comprehensive treatise of the theoretical aspects of volumetric analysis will interest chemists engaged in developing new methods of analysis or adapting old methods to meet new demands. The theoretical discussions are designed to aid the analyst in the critical examination of proposed reactions to determine their suitability for quantitative titrations. However, the authors emphasize the fact that only by careful investigation can theoretical deduction be put to test.

This book will be a worthy addition to the library of the analytical chemist, and most certainly should be of use as a text or reference book for courses in advanced analytical chemistry.

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of the

Thomas Burr Osborne Medal

to

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THE THOMAS BURR OSBORNE MEDAL

CHARLES G. FERRARI

We are in attendance at the twenty-eighth annual meeting of our Association, and this is the occasion of our annual banquet. The banquet is an affair that members look forward to. Not in many years has it required the promotion that was necessary earlier in order to insure its success. This evening the occasion has added significance and interest by virtue of the fact that the American Association of Cereal Chemists is honoring a distinguished scientist and educator by awarding him the Thomas Burr Osborne Medal.

The functions of a scientific society are many. Some are of considerable importance to the membership, but they are recognized and hence need not be enumerated. Our attention this evening is being focused on a function in which we take a great deal of pride. It was a happy thought indeed that matured into the establishment of the Osborne Medal Award. This function of our Association has dignity and far-reaching significance. The membership derives great satisfaction from doing it. People at large—because of the publicity attached to the award—are made aware of the contributions of a scientist to the progress of civilization. The scientific world notes the conferment of an honor upon a fellow scientist, and silently approves. In giving the medal, we elevate the plane of scientific achievement and indirectly benefit in so doing.

The Thomas Burr Osborne Medal was founded in 1926 when a committee headed by the late Dr. C. L. Alsberg was appointed. This committee established the basis of the award. This basis is expressed simply and succinctly on the face of the medal as follows: "For distinguished contributions in cereal chemistry."

The first recipient of the medal—Thomas Burr Osborne—had the medal named in his honor. It was conferred in Minneapolis in 1928. The second recipient was Clyde H. Bailey, to whom the medal was awarded at Detroit in 1932. Morris J. Blish received the third Osborne medal at Dallas in 1936. The last and fourth award was made to C. O. Swanson at Cincinnati in 1938. In recapitulation, the

years of the award have been 1928, 1932, 1936, and 1938. It cannot be said that the Osborne Medal has been awarded frequently. It can be said that it has been awarded judiciously. Osborne Medal Award Committees have guarded jealously the high standards set for the award, and the intent has been to make of the medalists a select company of distinguished scientists in the field of cereal chemistry or a branch of science related thereto. The Association as now constituted intends never to depart from that precedent and principle.

Your Toastmaster, as Chairman of the Osborne Medal Award Committee, takes pleasure in announcing a fact already published—namely, that by unanimous vote the committee elected Dr. Ross Aiken Gortner the fifth Thomas Burr Osborne Medalist.

Your Toastmaster derives no little satisfaction in the performance of his minor function on this occasion, because he is one of many who have been privileged to sit in Dr. Gortner's classroom, to listen to his inspiring lectures, to benefit from his counsel in scientific matters, and to know him as a very human and considerate gentleman. It would be interesting to see how many in this gathering have taken work at Minnesota in the Division of Agricultural Biochemistry of which Dr. Gortner is chief. Will those who have taken work there please rise? (It is estimated that more than fifty people stood up.)

At this point, I wish to introduce a friend and colleague of Dr. Gortner who, by virtue of his close and long association with the Medalist, is eminently qualified to tell us about him. I will now present Dr. Clyde H. Bailey, himself a recipient of the Osborne Medal, the first editor of *Cereal Chemistry*, and past president of this Association. He will tell us about the life and scientific attainments of the Medalist. Dr. Bailey.

THE CONTRIBUTIONS OF ROSS AIKEN GORTNER TO CEREAL CHEMISTRY

C. H. Bailey

In September, 1902, a youth named Ross Aiken Gortner entered the Academy of Nebraska Wesleyan University at Lincoln, Nebraska. His matriculation records showed that he was the son of the Reverend Joseph Ross Gortner, a Methodist clergyman, and Louisa Waters Gortner. He was born near O'Neill, Nebraska, and while still an infant he accompanied his family to the Garraway Mission station in Liberia. There the father had succumbed to a tropical malady, and later, with his mother and older brother, Ross returned to the family home in Nebraska. Despite the thin living in this prairie setting of those pioneer days, the indomitable spirit of the mother of Ross was never conquered. Determined that her sons should have the best education afforded by the region, and under the aegis of the church to which her husband had given his life, she drove her failing body to the task of caring for the home and of rearing her sons in the same determination for educational advancement.

It is doubtful, however, that Ross had the career of a chemist in mind when he came up to Nebraska Wesleyan. There are indications that his interests of the moment were in the field of the classics, and, in fact, this is an interest which he never lost. But in the early stages of his academy and college work he met a man who profoundly affected his student mind. This man taught chemistry, mineralogy, and geology in the college, and chemistry in the academy. Although Gortner was an academy, or college preparatory student, he actually entered some college classes, including elementary chemistry, and here he sat under the professor to whom reference has been made, Dr. Frederick Alway. The rigorous, scientific discipline to which the students in his classes were subjected appealed to the mind of young Gortner, who eagerly sought opportunity to advance his educational processes in this field. By working up courses out of class he qualified during his second year for a laboratory assistantship, and his name was carried over that title on the stationery of the department. Dr.

Alway commented to me that from that time forward, until he transferred to the University of Nebraska, Gortner almost lived in the laboratory except when he was at home with his invalid mother.

In fact, Gortner was confronted about this time with faculty regulations which forbade registering in as many credit hours of chemistry as he desired to carry. Despite these formal regulations, he did the work of certain courses in which he was not registered, and, in fact, kept ahead of the class in organic preparations sufficiently so that he could aid the regular students in his capacity as laboratory assistant.

Before the end of this year Gortner had fitted himself into certain phases of Professor Alway's research work. He prepared a new nitroso compound, after laborious fractional distillations, and developed new techniques that proved singularly useful in this phase of organic synthesis and purification. Consequently, during his junior year, he was the author of published scientific papers.

Professor Alway became a member of the faculty of the University of Nebraska at Lincoln in 1906, and while Gortner continued as a Wesleyan student until he graduated in 1907, it is a fact that he worked a substantial fraction of the time in Alway's laboratory at the University. This was the period during which Professor Alway was actively engaged in researches on bleached flour, and Gortner participated in those investigations. This was his first introduction to the field of cereal chemistry, a field not then recognized as a definite area of specialization among the several branches of chemistry.

Now Professor Alway was a graduate of the University of Toronto and so it was not surprising that, despite attractive offers from several universities, Gortner selected the University of Toronto as a place where he proposed to pursue graduate study. Here he came under the tutelage of Dr. W. Lash Miller, another rigorous disciplinarian insofar as science was concerned—one who brooked no half-way measures or inexactitudes in thinking or techniques. Miller was one of those rare geniuses of his time who bridged certain of the gaps between the physical and biological sciences. Although labeled a physical chemist, he did brilliant work in the study of bios, and those of you who attended the Toronto meeting of this association will recall his logical review of these researches. Thus another link was welded into this chain of educational processes to which Gortner was subjected in these formative years. Here at Toronto was inculcated, no doubt, an added respect for the physico-chemical approach to chemical problems and the effect of this indoctrination at the hands of Lash Miller

(whose teaching practices, so I have been told, suggested the appropriateness of this middle name by which he preferred to be known) has probably registered down through all the intervening years of Gortner's own researches.

After a year at Toronto, Gortner moved on to Columbia University, where he became a graduate school major under Dr. Marston T. Bogert. In one year's time, or only two years after his baccalaureate at Nebraska Wesleyan, Gortner qualified for his doctor of philosophy degree at Columbia in 1909. Bogert is an organic chemist, so the year at Columbia represented something of a shift of emphasis, thus providing a strengthening of the first link in this chain which was forged at Nebraska Wesleyan. Gortner once remarked to me that he virtually memorized Richter's *Organic Chemistry* during this year at Columbia, and I came to believe that that was no exaggeration, as disclosed by the almost encyclopedic knowledge which he could instantly command respecting details of structure and properties of organic compounds that most of us must ascertain by looking in the book.

At the time that he completed the requirements for the doctorate, Gortner was afforded an opportunity to become resident investigator in biological chemistry at the station for experimental evolution operated under the Carnegie Institution at Cold Spring Harbor, on Long Island, New York. This proposal was accepted, and he presently found himself associated with several distinguished biologists to whom he brought the chemical viewpoint in dealing with various fundamental issues of genetics, embryonic growth, pigmentation, and related fields. Thus was added another link in which things biological were of primary importance.

Five years were spent in this stimulating environment, until, in 1914 the suggestion was made to Gortner that he join the staff at the University of Minnesota. During the previous year the Division of Agricultural Chemistry and Soils of the Department of Agriculture at University Farm had been divided into two divisions. Dr. Alway had been persuaded to head the Division of Soils, and he selected as one of the members of his scientific staff the man who had been his former student and assistant at Nebraska Wesleyan. So the Gortner family, which at that time included a daughter and two sons, moved to St. Anthony Park and presently occupied the house on Raymond Avenue which is still the family home. Here was born a second daughter, Alice, the fourth and last child of this family.

After two years spent largely in the study of the nitrogen-containing compounds of Minnesota soils, Dr. Gortner was invited to move his activities across the hall into the Division of Agricultural Biochemistry. Professor Thatcher was head of the division at that time, but a year later, in 1917, he became Dean and Director of the Department of Agriculture, and Dr. Gortner became chief of the division, a position which he has occupied since that time, and thus, over a period of a quarter century.

As chief of this division, Dr. Gortner's interests have been many and varied. I was almost led to say "perforce" in the preceding sentence but decided, on second thought, that there was no perforce about it after all. For it is evident that his natural interests are wide and varied, and that he found, or made, opportunity to give them expression. In a university setting, it was inevitable that he should attract to himself a group of advanced and graduate students, who recognized his capabilities as a researcher and teacher and were eager to become a part of the thriving research institute of which he was the center. I shall not attempt here to recount all the details of this quarter century of activities, or even to list those portions of it which relate specifically to cereal chemistry. The latter will be suggested or disclosed by the bibliography which follows. Rather, I should like to attempt briefly to analyze what appears to me to be the fundamental philosophy of the developments along this line at Minnesota for which he was responsible.

Emphasis has already been laid upon the three links in the chain of training and education to which Dr. Gortner had been subjected prior to coming to Minnesota, namely, organic chemistry, physical chemistry, and applied biology. These, in turn, became essential parts of a new scientific structure which was recognized and named, and on which construction was begun at about that time, namely, colloid chemistry. Dr. Gortner was virtually present at the very beginnings of this new and highly significant departure from the conventional thinking of the previous period. Not only was his interest aroused but he became immediately active in the researches which brought distinction to him and his students. Early in this period he set into motion studies of water-binding capacity of wheat-plant tissues as related to cold-resistance and winter hardiness. These studies, and the methods which grew out of them, found wider application in many other fields of plant physiology, and with many and diverse plant forms. In certain of these studies, and particularly those which con-

cerned the vegetation of the western desert, and the distinction between tissue fluids of ligneous and herbaceous plants, he enjoyed the association of the late J. Arthur Harris. Dr. Harris was his colleague at Cold Spring Harbor, and later joined the faculty of the University of Minnesota as head of the Department of Botany. The friendship between Gortner and Harris was good to see. Here were two congenial spirits, both tolerant to the extreme when personal foibles and eccentricities of their fellows were concerned, but utterly intolerant of any compromise with scientific facts and scientific accuracy. Both drove themselves to the limit of physical endurance in their excursions into the field, and both found utter joy and satisfaction in these undertakings.

Wheat gluten and other proteins possessed colloidal properties that intrigued Gortner and around these substances were built researches that led to highly significant results. Parallel to these studies were the chemical investigations of the proteins for which the Gortner school was distinguished. This was an elegant combination of interests and one which yielded large returns.

It was indicated earlier that no attempt would be made to trace in detail all the investigations conducted by Gortner and his students during this period of a quarter century that he has been active in the field of cereal chemistry and related biochemical areas. I do wish to lay particular emphasis, however, on the sound scientific philosophy upon which they were based. In research and in teaching, the Minnesota school under Gortner's leadership has steadfastly held to the principle that if the fundamental phenomena are known and understood, the technological application will be made with greater exactness and definiteness. Students who have worked in this school have been held to a type of training which fitted that pattern.

The discussion of the attributes of a distinguished cereal chemist would not be complete without reference to the services which he has rendered to other branches of science. The list of such services is long, and only a selected portion of it can be mentioned here. Dr. Gortner has been a member of the National Research Council committees on colloids and on proteins, respectively, since their establishment. He served the sections of biological chemistry and of colloid chemistry of the American Chemical Society as secretary, and as chairman, and also served two terms on the editorial board of that Society. He was national president of the honorary chemical society Phi Lambda Upsilon from 1921 to 1926, president of the American

Society of Naturalists in 1932, and is now president of the Society of Sigma Xi, which is the largest scientific organization in the world. He was the Wisconsin Alumni Foundation Lecturer in 1930, Priestly Lecturer at Pennsylvania State College in 1934, and George Fisher Baker Lecturer at Cornell University in 1935–1936. The series of lectures at Cornell were published later in a useful volume entitled *Selected Topics in Colloid Chemistry.*

In addition to the many journal articles published by Dr. Gortner and his co-workers, which must number upwards of 350 by this time, he is the author of *Outlines of Biochemistry*, which is now in its second edition.

These and other activities and accomplishments have brought him many honors and distinctions, including membership and offices in the societies that have already been named, as well as in Gamma Sigma Delta, Phi Kappa Phi, Alpha Chi Sigma, Alpha Zeta, and the notable honor of election to membership in the National Academy of Sciences. The honorary degree of Doctor of Science was conferred upon him by Lawrence College in 1932.

No discussion of such a person as our medalist is complete or adequate without reference to his personal attributes. Also this is one of my pleasantest tasks on this occasion. For here at my right sits a man who has been a real friend to a great many people—students, professional colleagues here and abroad, and others. This friendship has taken the form of a kindly interest, aid in times of difficulty, a cordial welcome to the well-known home on Raymond Avenue, counsel concerning many technical and scientific matters, and a charming companionship on excursions of various sorts. We who have known him well and who have enjoyed the privilege of these associations through the past years have been singularly fortunate. The medal now awarded will itself be adorned by its association with a great scholar, a mentor of many of our craft, and a delightful friend.

PRESENTATION OF THE THOMAS BURR OSBORNE MEDAL

CHARLES N. FREY, *President*

The Osborne Medal Award is the highest honor that our organization can bestow. The Medal is awarded to those who, in the estimation of the Committee, have rendered highly important contributions to cereal chemistry.

As a philosopher, as a great teacher, and as a creative worker of extraordinary ability, you have developed and inspired a host of students who have studied with you, and have rendered distinguished service to your state and to our nation.

It gives me great pleasure to present to you, Ross Aiken Gortner, on behalf of the American Association of Cereal Chemists, the Thomas Burr Osborne Gold Medal.

ACCEPTANCE OF THE OSBORNE MEDAL BY DR. GORTNER

President Frey, Dr. Ferrari, Dr. Bailey, Members and Guests of the American Association of Cereal Chemists:

I am deeply appreciative of the tribute which has been expressed with regard to the things that I have been trying to accomplish during the past years and the fact that my efforts have found favor in the eyes of your Committee on Award as evidenced by the beautiful medal which has just been handed to me.

My gratification arises from two sources: In the first place, this medal bears the name of Thomas Burr Osborne and I regard Dr. Osborne as one of the greater of the scientists that America has produced. I cannot recall that I ever met Dr. Osborne in person but my personal correspondence file holds a number of letters that passed between us. Two of these letters I prize probably above all others in my personal file.

About 1911 when I was still associated with the Carnegie Institution of Washington I wrote Dr. Osborne and asked his advice as to whether or not I, as a young chemist, might not profitably undertake a study as to the origin of the black humin which was formed as a

resultant of the acid hydrolysis of proteins. Dr. Osborne replied to the effect that he felt it would be a waste of my time to attack this problem since older and more experienced chemists had already studied it without success.

Some years later in 1914–15 at the University of Minnesota one of your former medalists, Dr. Blish, then a graduate student, and I discussed this problem and we attempted to see if we could find a clue. This study was so satisfactory that the paper which resulted was followed by a series of other papers leading to the elucidation of the general phenomena which are involved in humin formation. When one of these later papers had appeared in print I one day received an entirely unsolicited letter from Dr. Osborne, a portion of which read essentially as follows:

> "Some years ago if my memory serves me correctly I advised you not to undertake researches on the origin of the humin problem. Allow me to congratulate you that you did not take my advice."

To me this letter is a precious document since it illustrates the greatness of both Osborne the man, and also Osborne the scientist.

My second reason for gratification tonight lies in the fact that the citation emphasizes my contribution as a teacher. Here perhaps I have found my greatest satisfaction. Whatever I have done or may do in the way of research may eventually have to be done over or done better by someone else as new methods and techniques become available. I have had great joy in helping the stream of knowledge to move ahead but the final evaluation of the importance of any or all of my scientific contributions must await the verdict of the historian of the future.

The training of students who will carry on in scientific fields is, however, a tangible item in which I take great pride from having had a part. Many of my former students are here tonight and I am sure that I get my greatest satisfaction in knowing that many of these hold high positions in your Association and that they will carry on in the field of science after I am no longer with you, and perhaps I may also claim a place in the scientific genealogy of their students and their students' students who will keep the blood lines of science flowing as the years go by.

One's place in the world of men is usually conditioned by two factors—heredity and environment. One has to inherit certain types

of germ plasm in order to achieve. One's basic germ plasm is unalterable so that any credit which may accrue to me for innate ability must in reality be interpreted as a recognition of good basic stock in the ancestral lines of my father and my mother.

The development of one's inherent tendencies depends upon environment. Perhaps the most potent factor in my environment was the request that my father—a missionary dying in Africa—made to my mother, "If possible give the boys an education." It was not easy for the widow of a Methodist minister to fulfill that request but my mother did it in spite of all handicaps. Her life was spent in giving her children an opportunity and she endured privations and toil which today would seem unbearable in order to carry out her own and my father's wishes. Anything I may have accomplished should be credited to her. She has a large part in the beautiful medal I hold in my hand.

Dr. Bailey has named other potent forces in my environment. Prof. F. J. Alway, who guided my first steps in the science field, who gave me every encouragement, and who not only taught me chemistry but also a philosophy of life. The late Prof. W. Lash Miller, who insisted on perfection as nearly as it could be obtained, and on precision measurements. The "Torments of the Lash" were good for those that survived and there was something lacking in those who failed for "the Lash" was always applied when it was needed and only to the degree that it was needed. It was applied as a stimulus and not as a deterrent.

Prof. Marston Taylor Bogert and the late Dr. J. Arthur Harris also share with me tonight in this medal award. To these and other teachers I owe much, for through their influence my footsteps were guided in the paths that I have followed.

And lastly my colleagues and graduate students with whom I have been associated for more than a quarter of a century in the Division of Agricultural Biochemistry at the University of Minnesota must share in this occasion. Again environment enters into the picture. Cereal chemistry has been a tradition of the Minnesota Agricultural Experiment Station since it was founded in 1888. How then could a young man coming into such an environment fail to become interested in certain aspects of cereal chemistry, especially since Dr. Bailey was a member of the department when I joined it and has been my loyal colleague throughout these many years? It was perhaps the "line of least resistance" to undertake studies in the cereal field.

These studies have been conducted in large part in collaboration with graduate students and whatever merit the studies may possess must be shared with them.

The past quarter of a century has held much of joy for me but perhaps my greatest satisfaction is the map which hangs on the wall of my office where map pins portray the locations of our former graduate students who are contributing to the instruction of our youth, to the advancement of knowledge, to the progress of industry, and the making of a better world.

Dr. Gortner at Work in His Study

President Frey, I accept the Thomas Burr Osborne Medal of the American Association of Cereal Chemists not as a token of any personal achievement but rather as the representative of the spirit of the cooperative group of staff and graduate students with whom I have been associated in the Division of Agricultural Biochemistry of the University of Minnesota during the last quarter of a century. I sincerely thank you.

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THE PLANT PROTEINS

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Just when man first differentiated between the various components of his diet we will probably never know. It must have been at a very early date in human history that foods from flesh were distinguished from those from seeds or grains. This permitted a rough classification of foods into those having an animal principle and those having a vegetable principle. Presumably it was early observed that those foods having an "animal principle" readily underwent putrefaction, giving rise to foul odors and (much later) to an alkaline reaction, whereas products of plant origin could be stored for long periods of time and when conditions were suitable for decomposition underwent a fermentation with the production of acidic products. Fermented beers of various types were unquestionably in use in ancient Egypt and Mesopotamia at the dawn of written history.

Apparently the recognition of an "animal principle" in plant products must be attributed to Beccari, who was Professor of Medicine, Anatomy, and Chemistry at the University of Bologna. In 1728 he lectured before the Academy of Science of Bologna, and seventeen years later his lecture was abstracted in the newly founded Proceedings of this Academy. Dr. Bailey and Mr. Loenholdt (1941) have rendered science a real service in translating this classic paper.

Beccari separated wheat into an "animal" part which he called "glutinosum" or glue-like, which today we know as gluten, and a plant part, "amylaceum," which today we know as starch. These two fractions he distinguished from each other by the nature of the products which they formed on fermentation or putrefaction and by the products which each yielded on destructive distillation. The "animal" portion yielded alkaline products; the "plant" portion yielded products of acidic nature.

One can imagine Beccari's great surprise when he tested other seeds or grains, such as beans, barley, etc., and found that he could not by his simple washing techniques separate them into distinct "plant"

and "animal" fractions. His reviewer notes that he "marveled at so great an unlikeness in similar things," and I may add that even in the year of our Lord 1942 we still marvel at the fact that the proteins of wheat form this all-important gluten.

Apparently Beccari's gluten was generally accepted as the generic name for all plant proteinaceous materials, and in fact later workers seemed to believe that there was only one plant product having such characteristics. Sir Humphry Davy (1813), in his celebrated lectures on agricultural chemistry before the Board of Agriculture, gives an almost modern description of the preparation of gluten from wheat flour and then goes on to credit Joseph Louis Proust (1754–1826) with the discovery of "gluten" in a great variety of plant products:

> "Gluten may be obtained from wheaten flour by the following process: the flour is to be made into a paste, which is to be cautiously washed, by kneading it under a small stream of water, till the water has carried off from it all the starch; what remains is gluten. It is a tenacious, ductile, elastic substance. It has no taste. By exposure to air it becomes of a brown colour. It is very slightly soluble in cold water; but not soluble in alcohol. When a solution of it in water is heated, the gluten separates in the form of yellow flakes; in this respect it agrees with albumen, but differs from it in being infinitely less soluble in water. The solution of albumen does not coagulate when it contains much less than 1000 parts of albumen; but it appears that gluten requires more than 1000 parts of cold water for its solution.
>
> "Gluten when burnt affords similar products to albumen and probably differs very little from it in composition. Gluten is found in a great number of plants; Proust discovered it in acorns, chestnuts, horse chestnuts, apples, and quinces; barley, rye, peas, and beans; likewise in the leaves of rue, cabbage, cresses, hemlock, borage, saffron, in the berries of the elder, and in the grape. Gluten appears to be one of the most nutritive of the vegetable substances; and wheat seems to owe its superiority to other grain, from the circumstance of its containing it in larger quantities."

Proust, however, later (1817) recognized that the "gluten" of barley was different from the "gluten" of wheat and gave it the name, "hordein." This appears to be the first instance in which differences in the nature of plant proteins was recognized. He must also be credited with the discovery and characterization of the first amino-acid to be isolated, leucine.

Proust was possibly guided and influenced by the studies of Fourcroy (1789) who demonstrated the presence of a coagulable protein in the juices of various plants and who prepared and studied a number of preparations of this product from various plant sources. He notes that this material has the characteristics and properties of animal albumins, and apparently he regarded this as the animal-like portion of plants.

The term ***protein*** (meaning pre-eminence), was first used by Mulder (1838) to designate those complex nitrogenous materials which were essential constituents of both plants and animals. Mulder believed that there was only one protein but that this combined with sulfur and/or phosphorus in different ratios to account for the various properties that the proteinaceous complexes exhibited.

Einhof (1805) is usually credited with the discovery that wheat gluten contains an alcohol-soluble fraction. However, it appears that Einhof did not realize the importance of his observation and did not recognize that he was making a fractionation of gluten into different proteins. He seems to have interpreted his results as indicating that gluten was appreciably soluble in alcohol and thus was only further characterizing the gluten as such.

It remained for Taddei (1819) to recognize that alcohol did produce a fractionation of the gluten and to give to the alcohol-soluble fraction the name gliadin. The residue, insoluble in alcohol, he designated "zymon." Apparently Taddei recognized and distinguished a third component, the water-soluble "albumin." If so, he must be credited with being the first investigator to clearly recognize the existence of more than one plant protein. That his views were not generally known or accepted is evidenced by the later assertion of Mulder that only one primary protein existed.

The confirmation of a water-soluble plant albumin in addition to Taddei's gliadin and zymon was apparently made by Berzelius (1827, 1828a, 1828b). Since Berzelius is noted for his great activity in analyzing all sorts of materials, it is not surprising to find this father of analytical chemistry studying plant products, and further since his laboratory was attracting students from all of Europe, his views were widely and rapidly disseminated.

With no new techniques available for the study of plant proteins it is not surprising that these views persisted for many years. As late as 1841, Liebig reopened the question of the plant proteins and came to the conclusion that there were only two distinctive proteins, Taddei's

gliadin which Liebig renamed "plant gelatin" and Taddei's zymon which Liebig called "plant fibrin." Here again one can see the influence of the "animal principle" idea since gelatin and fibrin were characteristic animal proteins. Liebig states that the soluble albumin of Berzelius is simply a more soluble modification of the plant gelatin.

However, a new technique was now introduced, the method of determining nitrogen by organic combustion, and on the basis of this technique Dumas and Cahours (1843) announced that wheat contained *four* distinct proteins, a "plant fibrin" which was insoluble in alcohol, a "plant casein" which separates from a hot alcoholic solution on cooling, a "glutin" which is alcohol soluble, and a water-soluble albumin. Again I would like to call attention to the new name which has been introduced, a "plant casein," again emphasizing the animal nature of the product.

The introduction of methods for the analysis of organic compounds by Dumas and Liebig provided investigators with potent new tools for protein research. The succeeding period culminated in the publication by von Bibra in 1860 of his celebrated *Die Getreidearten und das Brod.* In this volume of 502 pages von Bibra brings together both the technological and scientific information which was available on wheat, rye, barley, oats, rice, kafir, millet, maize, and buckwheat and includes many analyses and observations which he made himself in his own laboratories. This volume apparently represents the only excursion which this versatile scientist [1] made into the cereal chemistry field. It must have exerted a profound influence on the agricultural scientists of that day, for a reprint [2] was necessary in 1861.

[1] Baron Ernst von Bibra, M.D., Ph.D. (1806–1878) was orphaned as a child and inherited a large fortune. He graduated from the University of Würzburg and then devoted himself to chemistry, natural science, and extensive travel. Apparently he had his own private laboratory and was not associated with a university group. He resided at Nuremburg. His publications exist largely if not wholly in the form of books. Among the more important of his books mentioned by biographers are *Chemische Untersuchungen über die Knocken und Zähne der Menchen und der Wirbeltiere* (1844); *Chemische Fragmente über die Leber und die Galle* (1849); *Die Bronzen und Kupferlegierungen der Alten und altesten Völker* (1869); *Reisen nach Sudamerika*, 2 vols. (1854); *Erinnerungen aus Sudamerika*, 3 vols. (1861); *Aus Chile, Peru und Brazilien* (1862); *Reiskizzen und Novellen*, 4 vols. (1864); *In Sudamerika und in Europa*, 2 vols. (1874). Mention is made of other works on snake poisons, phosphorus poisoning, action of various drugs and questions of hygiene.

Oddly enough his *Getreidearten und das Brod* is not mentioned in the biographical sketches from which the above information was gleaned (*New International Encyclopedia, Encyclopedia Americana*, Lippencott's *Pronouncing Biographical Dictionary*). He is not mentioned in the *Encyclopedia Britannica.*

[2] The title page says "Second Edition." It is, however, a word-for-word reprint of the 1860 volume with no additions or subtractions. Evidently the same type (or plates) was used for the reprinting.

Von Bibra utilized extensively the ultimate analysis techniques of Liebig and Dumas for the protein preparations which he separated. He also analyzed many samples of grain for moisture, nitrogen, fat, sugars, gums, starch, and ash. In many instances the ash is analyzed for the various ash constituents. Wheats from Germany, England, Russia, Spain, Algiers, Egypt, and Australia were included in his studies, and in some instances these were subdivided into the spring and winter types.

Von Bibra recognized four wheat proteins, a water-soluble albumin, a plant fibrin, a plant gelatin, and a plant casein, thus adopting Dumas' and Cahour's classification and terminology.

The next major advance in the field of plant proteins may be attributed to Ritthausen, who was Professor of Agricultural Chemistry at the University of Bonn. Ritthausen began to publish on plant proteins in 1866, in which year he discovered glutamic acid. In 1868 he discovered and characterized aspartic acid. He was the first investigator to systematically utilize *acid hydrolysis* (sulfuric acid followed by removal of sulfate with lime) as a tool in protein research.[3]

Ritthausen analyzed proteins systematically for tyrosine, leucine, glutamic acid, aspartic acid, and leucine amide and records that all attempts to isolate other products from the gummy residue resulted in failure. His observations were collected and coordinated in his *Die Eiweisskörper* in 1872. In so far as the writer is aware this is the first book to be devoted exclusively to a discussion of proteins, and interestingly enough the proteins which he discusses are exclusively of plant origin. Approximately one-third of the volume is devoted to the proteins of wheat and spelt. Again he separates the wheat proteins into four entities, plant gelatin or gliadin, plant fibrin, casein, and "mucedin." The mucedin is the water-soluble protein remaining in solution after separation of the gluten, and in the dilute aqueous alcohol after separation of the gliadin. It is heat-coagulable and probably represents the fraction that others have called albumin. Ritthausen credits de Saussure (1833) with naming this fraction, mucin, and with noting that it acts strongly on starch, yielding reducing sugars.

[3] Braconnot (1820) was the first investigator to use acid hydrolysis to break down proteins. By boiling gelatin and flesh with dilute sulfuric acid he isolated and identified glycine. This was the first amino acid to be isolated from a protein hydrolysate. Unfortunately Braconnot's observation was not recognized as the introduction of a new and powerful tool into protein research, and the technique of acid hydrolysis remained essentially unutilized until resurrected by Ritthausen.

The next major advance in techniques which enabled chemists to more adequately characterize proteins came from the studies of Emil Fischer, which covered the period 1889–1918. His introduction of the ester method for separating amino acids, together with Hausmann's nitrogen distribution method and Kossel's studies of the basic amino acids, provided new and powerful tools which were almost at once applied to the field of plant proteins.

Almost simultaneously with the beginning of Fischer's work a young American chemist took up the mantle that Ritthausen had laid aside and began a life service in the study of plant proteins, utilizing the new techniques which had been developed. Thomas Burr Osborne published prolifically in this field from 1891 to 1929 and became internationally recognized as the world's foremost authority on plant proteins. For many years Fischer's laboratory in Berlin and Osborne's laboratory at the Connecticut Agricultural Experiment Station were recognized as the sources of authority on techniques and methods in the field of protein research. Fischer roamed widely over the protein field but apparently accepted Osborne's papers at face value and left the field of plant proteins largely to his distinguished American colleague.

In his later years Osborne collaborated first with H. Gideon Wells, of the Department of Pathology of the University of Chicago, in studies of the immunological behavior of plant proteins and later with Lafayette B. Mendel, of Yale University, in studies of the nutritive properties of the plant proteins.

Osborne's contributions to cereal chemistry will forever be inestimable, and it is altogether fitting that the medal of the American Association of Cereal Chemists should be established in his honor. His mastery of the techniques of the difficult Fischer ester method probably exceeded that of any other investigator working outside of Fischer's laboratory and equaled that of the best of Fischer's own technicians.

With Osborne's critical studies the available techniques were utilized to their maximum capacity, and further advances in the plant protein field had to await new viewpoints and new techniques. For a time it appeared that the field had been worked out and that all that remained was to introduce new refinements in old techniques.

In 1908 the protein field had been so thoroughly surveyed that a joint committee of the American Society of Biological Chemists and

the American Physiological Society [4] prepared a scheme of protein classification, which scheme is in general use even today. In this classification the plant proteins are assigned to (1) the albumins, (2) the globulins, (3) the prolamines, and (4) the glutelins. It is noteworthy that two of these classes, the prolamines and the glutelins, are limited to plant proteins. At last we have gotten away from the idea that plant proteins are wholly of an animal nature!

The criteria upon which this classification was based were largely physical properties, particularly "solubility" in various reagents under somewhat poorly defined conditions. For example, the globulins were defined as insoluble in water or aqueous alcohol but soluble in "dilute" salt solutions, whereas the glutelins were insoluble in these media and soluble only in the presence of dilute mineral acids or alkalies. At the same time it was recognized that globulins undergo a protein $\leftrightarrows$ protean transformation whereby the salt solubility is lost, the resulting product having acquired all of the properties of a native glutelin. Nevertheless the altered globulin is placed in a "derived protein" class rather than being considered as a glutelin.

Apparently the first persons to question the strict application of solubility techniques to protein classification were Gortner and Hoffman (1927) who noted that Osborne had never prepared all of the proteins from a *single* sample of wheat flour. In an attempt to see how the proteins of a single sample of flour would fractionate, Gortner and Hoffman used somewhat different techniques from those earlier employed by Osborne, in that the "salt-soluble" proteins were extracted with 5% potassium sulfate solution rather than 10% sodium chloride. Inasmuch as the results differed markedly from those expected from a reading of Osborne's papers the work was repeated using 10% sodium chloride solutions, and thereby results more nearly conforming with those of Osborne were obtained. This at once raised the question as to what salts in what concentrations one should use to extract the water- and salt-soluble albumins and globulins.

Accordingly a series of twelve different wheat flours were extracted with a series of twenty-seven different salt solutions, in most instances in four different concentrations. This study (Gortner, Hoffman, and Sinclair, 1928) revealed the fact that each flour in each different salt solution yielded a different percentage of salt-soluble proteins, and furthermore the percentage of salt-soluble protein in each flour differed

[4] Joint Recommendations of the Physiological and Biochemical Committees on Protein Nomenclature, J. Biol. Chem., 4: xlviii–li (1908).

with each concentration of each salt solution. It accordingly became evident that we were not dealing in all cases with true albumin and globulin fractions which exhibited true solubility but rather with a colloid system which under the influence of the varied ionic environment possessed varying degrees of peptizability. This was especially noticeable in the series of molar solutions of the potassium halide salts where the KF solutions peptized an average of 13%, the KCl solutions an average of approximately 23%, the KBr solution an average of approximately 35%, and the KI solutions an average of approximately 65% of the total protein. All of these solutions had earlier been adjusted to a common pH value, so that variations in pH were not a factor. All known data were conclusive in the demonstration that normal wheat flours contain far less than the 23% of salt-soluble proteins extracted by the NaCl solution, to say nothing of the 65% of total protein extracted by the KI solution. Here again each of the twelve flours studied showed its own characteristic behavior, so that the range within the series of twelve flours, for any particular salt solution, formed an almost continuous series from that of the halide of lower atomic weight to the halide of next higher atomic weight. (*Cf.* Gortner, Hoffman, and Sinclair, 1929.)

These studies then demonstrated that insofar as the proteins of wheat are concerned, we were not dealing with true solubility effects in determining the "salt-soluble" protein fractions and focused attention on the colloidal behavior of the wheat protein complex.

In a continuation of these studies Staker and Gortner (1931) extended the salt peptization theory to a large series of meals from various seeds and grains, representing most of the materials which Osborne and coworkers had used in their protein studies. Here again certain seeds and grains exhibited the most varied behavior toward salt solutions. Certain of the data are shown in Figures 1 and 2. It will be noted in Figure 1 that the *Triticum* species show in general rather uniform behavior, although in the samples studied there were detectable differences in actual amounts of protein peptized. Other seeds and grains showed somewhat similar tendencies, while still others differed widely from the *Triticum* series. Noteworthy is the essential lack of ionic effects in the case of the protein of Brazil nuts and, as Saunders (1931) and Rotha and Saunders (1932) have shown, in the case of the protein of the seeds of citrus fruits. In these cases we seem to be dealing with a true solubility of the crystalline globulins which

characterize these species. The same may be true of the proteins of the *Cruciferae*, where again the ionic effects are at most very slight.

Parallel with the above studies Sörensen was studying the solubility behavior of protein systems in the Carlsberg Laboratories. His studies led him to the reversible-dissociable component theory enun-

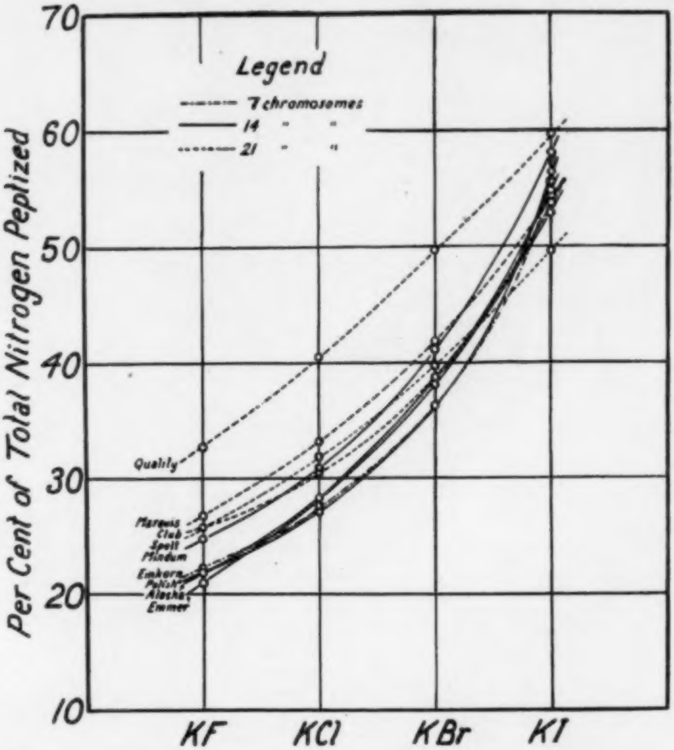

Fig. 1. Peptization of proteins of the meals from various *Triticum* species by $0.5M$ salt solutions.

ciated in 1930. In brief he suggests that proteins as isolated comprise a series of long-chain polypeptides, each of which has its own characteristic solubility and other physico-chemical behavior. The integrated effect of all of these individual characteristics is the behavior which one measures in the protein system under examination. Sörensen suggested that the original protein complex $Ax\ By\ Cz \cdots$ might be capable of fractionation into x units of A, y units of B, and z units of C, etc., and that in this way the physico-chemical properties of solutions of fractions A, B, and C would each be found to differ mark-

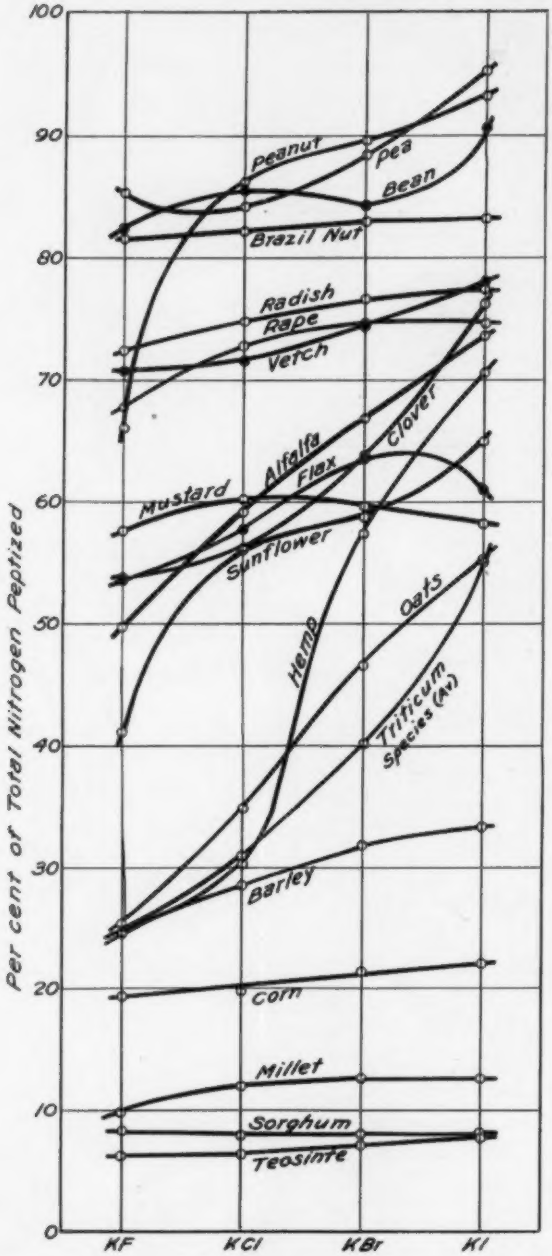

Fig. 2. Peptization of the proteins of the meals from various seeds and grains by 0.5*M* salt solutions.

edly from each other and all would differ from the original *ABC* complex. He further suggests that if the isolated fractions *A*, *B*, and *C* are then recombined in the ratios x, y, and z in which they originally occurred, then all of the physico-chemical properties of the original complex would be regained.

This theory assumed true solubility behavior of the individual components and an additive effect of properties when two or more components were simultaneously present in solution. On the other hand the theory of Gortner, Hoffman, and Sinclair, emphasizing colloidal properties and differential peptizability by ionic solutions, laid primary stress upon surface forces (adsorbability of ions) and minor stress upon amino acid differences in the polypeptide chains making up the protein mass. This latter theory assumed that marked differences in peptizability and other physico-chemical behavior might result from the environmental conditions under which the protein aggregate of the endosperm was laid down in the wheat kernel even though the amino acid composition and protein chain length were essentially similar in all cases.

In order to test further the colloid theory, Sinclair and Gortner (1933) studied the peptizability of gliadin by inorganic salt solutions. It has been increasingly evident that the large amount of protein extractable from wheat flours by molar solutions of KI could arise only from the true gluten proteins, and it early appeared that gliadin was the gluten protein which was being peptized. Accordingly a quantity of gliadin was prepared by the classic alcohol method, and the dry protein was subjected to successive extractions with aqueous solutions of KI. It was observed that approximately 65% of a given gliadin preparation could be "dissolved" (peptized) by the KI solution, whereas approximately 35% remained "insoluble." That portion which was peptized formed a perfectly clear sol which exhibited no trace of opalescence. The original gliadin was thus fractionated into "soluble" and "insoluble" fractions. According to Sörensen these should be different components having different chemical composition, which chemical composition would account for their different "solubilities." The physico-chemical properties of the original gliadin preparation should accordingly be regained by a recombination of these fractions.

The two fractions were now freed from KI by dialysis and electrodialysis and reworked through solution in alcohol, *following as nearly as possible the original technique for the preparation of the original dry*

gliadin sample. We now had two preparations, one of which had been completely "soluble," the other essentially completely "insoluble" in aqueous KI solution. These two preparations were then individually extracted with aqueous KI solutions. Both preparations were found to exhibit essentially the same behavior as the original preparation from which they had been fractionated; *i.e.*, both the reworked KI "soluble" fraction and the KI "insoluble" fraction now were peptized to the extent of approximately 65% with an approximately 35% "insoluble" residue. Here the original properties had been regained not by a recombination of fractions as Sörensen's theory demands but rather by a reworking so that each preparation attained essentially the same colloidal state as that which characterized the original preparation. This experiment, therefore, supported the viewpoint that the environmental conditions which surround the protein as it is being laid down in the endosperm, together with conditions which prevail during harvesting and storage, may have a profound influence on the physico-chemical properties that the protein will exhibit in subsequent treatments.

These and other studies have pointed in this direction: that perhaps in any given biological structure there may not be the great variety of proteins which we can prepare from that structure by fractionation procedures but rather there may be a single protein complex to which we must ascribe the special properties which that structure exhibits. This protein complex we can fractionate into fragments by various techniques, but these fragments may be in a large measure artifacts, formed by our fractionation procedures. This would be especially true if surface phenomena, such as characterize colloidal systems, are of major importance in determining physico-chemical properties.

This view that perhaps the protein complex of the wheat kernel is not arbitrarily subdivisible into an albumin (leucosin), a prolamine (gliadin), and a glutelin (glutenin), to mention only the three on which all workers seemed to be agreed, is evidenced by the studies of McCalla and Rose (1935) who dispersed gluten in solutions of sodium salicylate and then fractionally precipitated this sol with magnesium sulfate. Figures 3 and 4 show certain of their data. It will be observed that the fractionation curves are essentially smooth curves with no breaks, indicating a shifting from a glutenin to a gliadin, and likewise the analytical data form essentially smooth curves with no sharp discontinuities. The changes in chemical composition which they found would seem to indicate an almost continuous series of polypeptide

chains, each differing very slightly from the adjacent ones in chemical composition and the whole forming a single colloidal protein complex having the properties of gluten. We know full well that the properties of gluten as isolated from a sample of wheat flour are not the additive

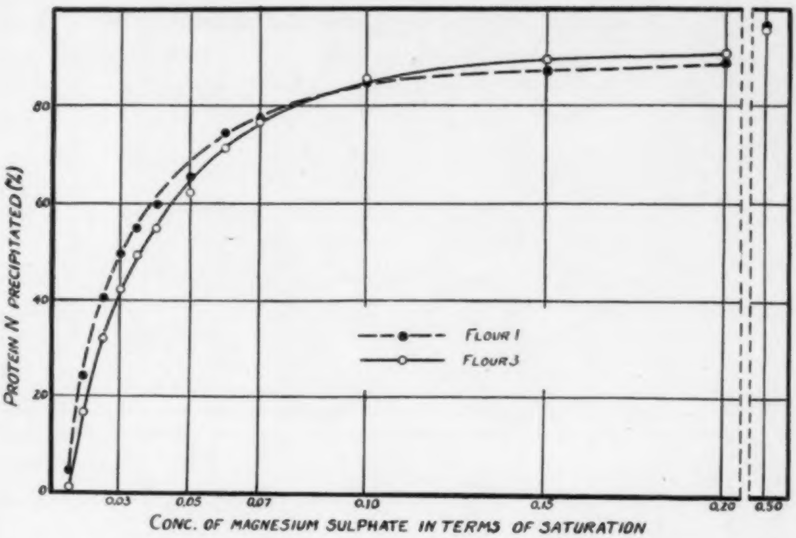

Fig. 3. The precipitation by various concentrations of magnesium sulfate of wheat gluten dispersed in sodium salicylate solutions (data of McCalla and Rose).

properties of preparations of gliadin and glutenin which we can prepare in the laboratory from the gluten, and perhaps it would be wiser to focus our attention more upon the gluten itself than upon any particular fractions that we can prepare from it.

This view that gliadin and gluten, the old plant gelatin and plant fibrin, may not be chemical entities but may be either mixtures or artifacts formed by laboratory techniques is supported by Rich's (1936) observations where conclusions similar to those of Gortner *et al* and McCalla and Rose are reached.

Later studies by McCalla and Gralén (1940, 1942), using ultracentrifuge techniques on the gluten protein dispersed in sodium salicylate solutions, lead to the conclusion that gluten contains a great many components which vary regularly and systematically in their chemical and physical properties. These authors conclude that there are present protein chains of many different lengths and that the dissymmetry of the molecules increases as "solubility" decreases. These

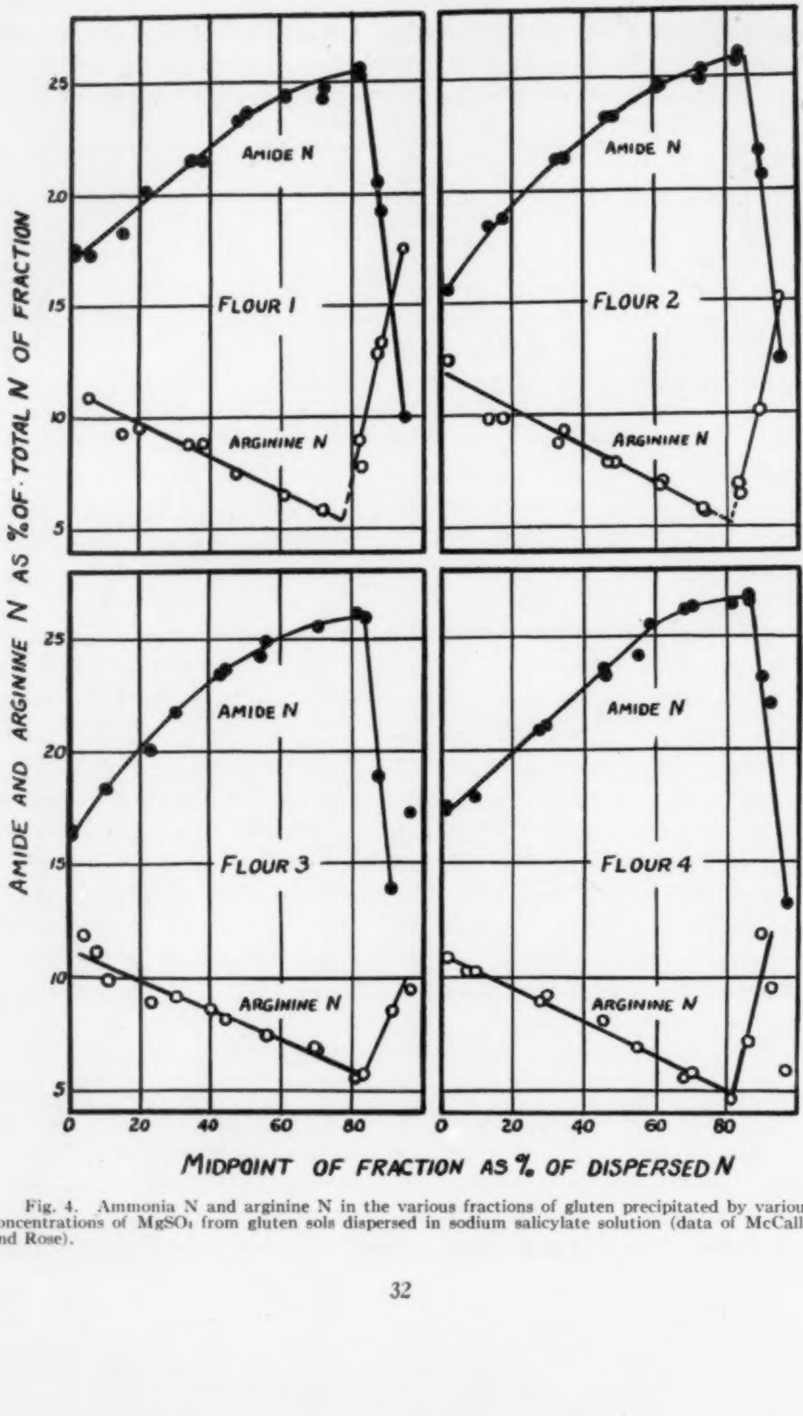

Fig. 4. Ammonia N and arginine N in the various fractions of gluten precipitated by various concentrations of $MgSO_4$ from gluten sols dispersed in sodium salicylate solution (data of McCalla and Rose).

studies and others support Sörensen's theory of a large number of distinct components in many of the systems which we had earlier believed to be fairly well defined.

Thus the newer physico-chemical techniques have to some extent at least altered our complacent assurance that we know the whole story of the nature of the proteins of the wheat kernel. The story is just beginning to unfold. New techniques will add more and more, and often in the future we shall have to revamp our ideas as new techniques are added.

On the horizon at the moment is another new technique, the Tiselius method of electrophoresis. This method has been applied practically not at all to studies of plant proteins. Later in this morning's program a preliminary report of its use with solutions of gliadin will be presented. I do not wish to anticipate the presentation of that paper except to say that the method apparently indicates that gliadin as usually prepared is far from homogeneous and contains a large number of electrochemically different components. If, as we hope we may be able to do, we can separate these different electrochemical components and subject these separately to various types of analytical study, we may have advanced one rung farther up the ladder of our knowledge of the wheat proteins.

Twenty-five years ago I knew and taught as facts many things with respect to proteins and protein behavior. Much that I knew and taught then has since been shown to be in error or to be half truths. Today I am much less sure of what I *know* about proteins than I was then, but I do know that they present some of the most fascinating and at the same time exasperating problems that can attract an inquiring mind. They present a multitude of problems that still await solution. New techniques must be devised and old ones improved. From it all as the years go by and workers multiply we may be able to account for the order which characterizes these pre-eminent compounds of biological organisms.

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